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Hazard Indices and Human Health Risks Associated with Toxic Element Contaminants in Bivalve Shellfish from Niger Delta, Nigeria

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Abstract

The quality of coastal waters in Niger delta have increasingly and adversely impacted by varieties of contaminants occasioned by environmental degradation and aquatic perturbation posed by petroleum exploration activities. This tends to undermine nutritional and health benefits derived from consumption of shellfish harvested from these waters. This study investigated tissue burden, hazard indices and human health risks associated with toxic element contaminants in bivalve shellfish harvested from coastal waters of Niger delta. Four species of bivalve shellfish; bloody cockle (*Anadara senilis*), donax clam (*Donax rugosus*), knife clam (*Tagelus adansonaii*) and mangrove oyster (*Crassosstra gasar*) collected from four locations were assessed for levels of toxic element contaminants as well as hazard indices and human health risk associated with their consumption. The tissue burden of toxic element contaminants was determined using atomic absorption spectrometer while United State Environmental Protection Agency (US EPA) method was employed to estimate hazard indices and human health risk. Results indicated lead concentrations were within the 1.5mg/kg acceptable limits while levels of cadmium, arsenic and mercury were higher than FAO limits of 0.5, 0, 0.5 mg/kg respectively. The estimated human health risk indicated non-carcinogenic values and hazard indices higher than threshold value of one for cadmium, total arsenic and methyl mercury while values for inorganic arsenic at some locations were higher than stipulated one in one million ($1.0x10^{-6}$) chances. This implies that toxic elements apart from lead in bivalves shellfish from these locations can induce potential deleterious health effects at consumption of 48g/day of bivalve shellfish.

1. Introduction

Food contamination by chemical contaminants have continued to be a subject of serious concern to researchers globally particularly due to their harmful effects on human body. Among the chemical contaminants are toxic elements which constitutes food safety risk because of their poor rate of metabolism, potential to bioaccumulate in aquatic ecosystems resulting from their non-biodegradable nature and long biological half-lives **(Censi *et al.*, 2006)**. Although some toxic elements occur naturally in the environment, anthropogenic inputs which originate from various human activities have continued to increase their concentrations **(Sarkar *et al.*, 2008; Giri *et al.*, 2015)**. Also, increased coastal population, rapid urbanization, oil and gas production, artisanal petroleum refining, oil spillage, tourism development, heavy rainfall throughout, and other economic activities have created numerous environmental and ecological problems in the Niger delta coastal areas **(Ukwo *et al.*, 2019)**.

Marine bivalve shellfish such as clams, mangrove oyster, cockles and other benthic filter feeders organisms are found on the mangrove mudflats, intertidal sandy beaches and the estuarine waters of the Niger delta. Bivalve shellfish are usually exploited by adult female and youth of these coastal communities **(Udotong *et al.*, 2017)**. Bivalve shellfish and other seafood products are currently the cheapest source of animal protein consumed by the average Niger delta and it accounted for about 50% of the total protein intake. Bivalve shellfish are also economically and nutritionally very important for human consumption, and particularly playing a central role in the Niger delta gastronomy. Apart from forming greater part of diet to the coastal population, bivalve shellfish constitute both traditional and primary source of enterprise and livelihood to most population of these communities **(Ukwo *et al.*, 2019)**. Seafood including bivalve shellfish have always contained certain amounts of toxic elements as a consequence of their habitat. In open seas, unaffected by pollution, bivalves mostly carries just the natural burden of toxic elements content. However, in polluted areas which have no sufficient exchange with the world oceans, in estuaries, in rivers and especially in places which are close to sites of industrial activities, the concentrations of toxic elements usually exceed the natural load **(Oehlenschläger, 2002)**. Most species of bivalve shellfish consumed in Nigeria are harvested from the brackish water that is exposed to varying amounts of chemical and environmental contaminants such as industrial chemicals, toxic residues from various anthropogenic

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activities. In the Niger delta region of Nigeria, pollution of the coastal waters has continued to attract greater attention. This is due to the high level of environmental degradation posed by petroleum production and exploitation along the coastline **(Wala *et al.*, 2016; Zabbey and Babantunde, 2015)**. Petroleum hydrocarbon from oils spills and human-mediated activities are usually incorporated into sediments where they can persist for years gradually releasing toxic substances into the immediate and remote environments **(Zabbey and Babatunde, 2015)**.

According to **Amnesty International (2018)** reports, Niger Delta region is the most recognized oil-producing region in Africa. It is also known to be the most polluted area on earth. The prevailing widespread pollution has severally impacted negatively on the food product especially seafood obtained from the coastal waters of this area. Also, the filtration nature of bivalves has led to accumulation of high amounts of some toxic elements, usually at higher concentrations than in what is obtained in the sediment making a bivalve a better indicator and thus, easily reaching toxic concentrations to themselves and their consumers **(Figueira *et al.*, 2011)**. Because the above stated reasons, levels of toxic elements in bivalve shellfish are of serious public health concerns, therefore the European Commission and other regulated bodies have set Maximum Permissible Limits (MPLs) for toxic elements in edible tissues of bivalve molluscs **(EC, 2006)**.

Some of the deleterious effects associated with dietary intake of these contaminants include diarrhea and gastrointestinal disorders, immune suppression, neurological disorder, reproductive impairment, developmental retardation, cardiovascular disorder, liver disease, infertility and miscarriage **(ASTDR, 2002; Ukwo *et al.*, 2019)**. The groups most vulnerable to dietary exposure of the contaminants are child-bearing women, children below twelve years, and subsistence fish farmers **(FAO/WHO, 2011)**.

For better understanding and characterization of the risk presented by chemical toxins in the environment to human and ecological receptors, most researchers used benthic organisms such as bivalve shellfish as bio monitors of the levels and long-term influences of chemical toxins within the ecosystem These circumstances make them important sources of food borne diseases which represent a significant health risk to consumers **(Sarkar *et al.*, 2008)**. The objectives of the present study are to determine the levels of toxic elements accumulated by bivalve shellfish harvested from the coastal waters of the Niger delta as well as estimate hazard indices and human health risk associated with toxic element contaminants in bivalve shellfish consumed in Niger delta. This study will assess their suitability for human consumption and provide baseline information on the quality and safety of fresh bivalve shellfish obtained from these coastal locations. The study will also quantify the potential human risk associated with bivalve shellfish consumption in the Niger delta.

2. Material and methods

2.1 Study Location

The location is a stretch of Atlantic coastline in the Niger Delta region of Nigeria. The Niger Delta sustains Africa's largest, and the world's third mangrove forest, bearing not only Nigeria's most abundant petroleum resources, but also diversified ecosystems, with numerous aquatic and terrestrial organisms **(Okonkwo *et al.*, 2015)**. Four locations along the Atlantic coastline of Niger Delta were chosen for this study: Andoni (4^{0}28' - 4^{0}45' and 7^{0}22'-7^{0}23), Bonny (4^{o}23`- 4$^o$25` and 7^{0}05'-7^{0}15'), Ibeno (4^{o}56' -4^{o}57` and 8^o 07' - 8^{o}15') and Iko Town (4^{o}20' – 4^{o}35' and 7^{o}40' - 7^{o}50'). Bonny and Andoni are located in Rivers State while Iko Town and Ibeno are located in Akwa Ibom State both in the Niger Delta region of Nigeria (Fig 1). The locations were chosen because of their accessibility and availability of the four (4) species of bivalve molluscs and also the fact that they served as important delicacy and food for indigenous people. They also served as an important source of income and employment for the people in these communities. The locations are essentially estuarine in nature with brackish water characterized by fine sandy beaches surrounded with mangrove swamp and intertidal mudflat in which Nypa vegetation dominate. The area is also naturally endowed with abundance of rivers, creeks and streams which received water and waste from the interland into the Atlantic Ocean. Also, this coastal environment has continued to suffer from environmental degradation occasioned by exploration and production of petroleum, liquefied natural gas production and spillage of petroleum products.

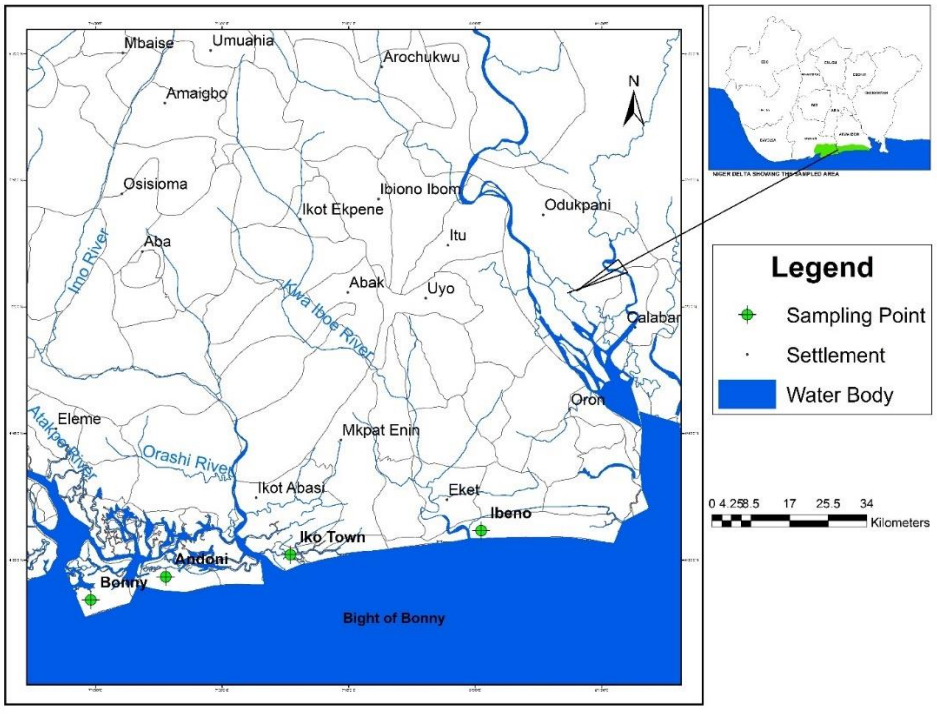

Figure 1. Section of Niger Delta coastal area showing the sampling locations along the Atlantic coastline. Insert Map: of the Niger Delta showing the study location

2.2 Sample Collection and Treatment

Fresh bivalve specimens mostly consumed in these localities were harvested manually by fishermen during low tide from intertidal estuarine mudflats of the different study locations. The bivalve specimens collected were, Bloody cockle (*Anadara senilis*), Donax clam (*Donax rugosus*), Knife or Razor clam (*Tagelus adansonii*) and Mangrove oyster (*Crassosstrea gasar*). They were identified at the Department of Fisheries and Aquatic Environmental Management, University of Uyo. At each sampling site, twenty (20) species of each bivalve specimens were collected and transferred to the laboratory within 24 hours of collection in plastic containers washed with 5% nitric acid and rinsed with distilled water before use. The traditional method of preparing bivalve for consumption was used in this study. At the laboratory, the bivalves were promptly cleaned of incrustations, washed in distilled water to remove all dirt, put into a stainless pot, and blanched for 5 minutes at 100°C. After blanching, the samples were poured into a perforated basket to drain and allowed to cool at room temperature (28±2°C). Samples were then shucked with sterile scalpel to extract the flesh into a sterile container. The extracted tissues were homogenized for 60s in a stomacher (Seward Laboratory Stomacher 400, England) and stored at -20°C in a scanfrost deep freezer for various experimental assays.

2.3 Analysis for Toxic Element Contaminants of Bivalve Shellfish

The levels of toxic element present in the bivalve samples were determined using a perkin – Elmer model 3030 Atomic Absorption spectrophotometer (AAS) at their respective lamp and wavelength. Standard stock solution of the element to be analysed were prepared, diluted to the corresponding working standard solution for recovery experiment according to the methods as outlined by **Onwuka (2018)**. The wet ashing method as outlined by **Onwuka (2018)** was used to determine the concentration of toxic element in the bivalve samples. The method of preparation and digestion procedure as outlined by **AOAC (2010)** for biological sample was also employed.

2.4 Human Health Risk Assessment Procedure

Assessment of human health risk for ingesting bivalve shellfish with toxic elements contaminants were determined based on methods outlined by **US EPA (2000)**. The non-carcinogenic risk due to consumption of chemical contaminants were determined using target hazard quotient (THQ) values as shown in the equation below:
Firstly, the level of exposure resulting from consumption of toxic element contaminants in bivalve samples can be expressed by estimating the daily intake levels.

$$EDI = \frac{C\ x\ IR\ x\ EF\ x\ ED}{BW\ x\ AT.}$$

Where
EDI = Estimated Daily Intake (mg/kg – day)
IR = Ingestion rate of Bivalve (kg/day)
C = Concentration of chemical contaminant in bivalve tissue (mg/kg)
EF = Exposure Frequency (days/year)
ED = Exposure duration (years)
BW = Body weight (kg)
AT = Average time (days)

Risk for both carcinogenic and non-carcinogenic chemicals were calculated in this study and the above equation was used as the intake equation.

The non-carcinogenic risk due to consumption of toxic element contaminants were then determined using target hazard quotient values (THQ).

$$THQ = \frac{EDI}{RfD}$$

Where:
THQ = Non-carcinogenic risk value of chemicals contaminants.
EDI = Estimated daily Intake or exposure rate (mg/kg –day)
RfD = Reference dose of chemical (mg/kg – day)

Secondly, Carcinogenic risk is express as a product of EDI and cancer potency value or cancer slope factor (CSf) and the following equation was used to estimate lifetime risk of cancer.

Risk = EDI* CSf

Where: Risk = Lifetime cancer risk
EDI = Estimated Daily Intake mg/kg-day
CSf = cancer slope factor mg/kg-day

The calculations were made using the standard assumptions for an integrated USEPA risk analysis **(US EPA 2009)**. For the purpose of this study, the intake rate of fish (IR) was assumed to be 48g/person per day (FAO 2017), average body weight of exposed individual (70kg), exposure frequency(365day/year) while duration of exposure was taken to be an average life expectancy of a Nigerian (55.20years) as reported by **WHO, (2018)**. The length of time for average does was calculated as 365days x 55.20 years. It was also assumed that ingested doses were equal to absorbed contaminants doses. For calculation of Target Hazard Quotient (THQ) for toxic elements, the following reference dose (RfD) as listed by **US EPA (2009)** were used for the respective elements; Pb = $3.6x10^{-3}$mg/kg-day, Cd = $1.0 x 10^{-3}$mg/kg-day, total arsenic = $3.0 x 10^{-4}$mg/kg-day and Hg = $1.0 x 10^{-4}$ mg/kg-day as methyl mercury (meHg). The hazard index (HI) is the sum of total hazard quotients; (HI = ΣTHQ). Carcinogenic risk is express as a product of EDI and cancer potency value or cancer slope factor (CSf) and the following equation was used to estimate life time risk of cancer. The carcinogenic risk value for inorganic arsenic was determined using the cancer slope factor (CSf) of 1.5mg/kg-day

2.5 Data Analysis

All the analyses were carried out in triplicate and data obtained from laboratory analyses were subjected to two-way analysis of Variance (ANOVA) to evaluate the effect of location and species on bivalve molluscs. Level of significance was set at $P<0.05$. Means with significantly difference were separated using Ducan-multiple Range Test. All experiments were conducted in triplicate and data were analysed using XLSTAT – Pro software program, Addinsoft, Boston (USA) Version 2018.7.

3. Results and Discussion

3.1 Toxic elements

The toxic elements content of bivalve shellfish species is presented in Table 1 and Figure 2. Results indicated significant differences ($p< 0.05$) in lead, cadmium, arsenic and mercury content across species and locations. The highest lead concentration of 1.68 mg/kg was recorded at Bonny location while the lowest value of 0.25 mg/kg was recorded at Iko Town in mangrove oyster. Analysis for cadmium indicated a concentration of 4.24 mg/kg in mangrove oyster at Iko Town

was the highest and was closely followed by knife clam with 3.74 mg/kg at Ibeno location. The highest concentrations of arsenic and mercury were recorded at Bonny location with 2.05mg/kg for arsenic while 1.34 mg/kg of mercury in knife clam at the same location. The total concentrations of toxic elements accumulated by bivalve shellfish in each sampling location are shown in Fig. 2. It indicated that bivalve shellfish samples from Bonny location had the highest toxic element accumulation and was closely followed by samples from Ibeno while samples from Andoni and Iko Town had relatively lower toxic element concentrations. The concentrations and levels of accumulation of toxic elements by bivalve samples in this study clearly revealed that bivalve shellfish are differentially selective for a range of toxic element and these variations might be influenced by a number of intrinsic (e.g. size, age, and sex) and extrinsic factors (e.g. metal speciation, Temperature and salinity). Also, the concentration of toxic elements in the tissue of marine invertebrates depends on the accumulation strategy adopted by each bivalve shellfish for each element **(Sarkar *et al.*, 2008)**. This strategy results from net differences between rate of absorption and excretion of elements, the permeability of the body surface, and the nature of the food and the efficiency of the osmo regulatory system present **(Benson *et al.*, 2017)**.

Table 1. Effect of location and species on Toxic metal content of bivalve (mg/kg)

Location	Species	Lead (Pb)	Cadmium (Cd)	Arsenic (As)	Mercury (Hg)
Andoni	Cockle	1.43±0.05^{bc}	1.40±0.47^{f}	0.85±0.12^{d}	0.61±0.10^{b}
	Donax clam	1.25±0.04^{d}	1.68±0.07^{ef}	0.40±0.02^{f}	0.53±0.03^{bc}
	Knife clam	0.63±0.09^{f}	2.04±0.16^{de}	0.12±0.05^{g}	1.19±0.02^{a}
	Oyster	1.04±0.15^{e}	0.78±0.08^{g}	0.82±0.02^{d}	0.58±0.06^{b}
Bonny	Cockle	1.54±0.07^{ab}	2.48±0.02^{c}	1.41±0.11^{c}	0.66±0.12^{b}
	Donax clam	1.63±0.06^{a}	1.37±0.05^{f}	2.05±0.09^{a}	0.52±0.02^{bc}
	Knife clam	0.98±0.05^{e}	2.25±0.07^{cd}	1.63±0.05^{b}	1.33±0.07^{a}
	Oyster	1.68±0.13^{a}	1.78±0.09^{ef}	1.56±0.03^{b}	1.34±0.02^{a}
Ibeno	Cockle	1.33±0.04^{cd}	0.60±0.22^{g}	1.40±0.02^{c}	0.11±0.10^{d}
	Donax clam	0.99±0.10^{e}	2.60±0.41^{c}	0.83±0.09^{d}	0.59±0.08^{b}
	Knife clam	1.24±0.04^{d}	3.74±0.38^{b}	0.66±0.17^{e}	0.66±0.36^{b}
	Oyster	0.45±0.08g	3.50±0.33^{b}	0.83±0.08d	0.38±0.12^{c}
Iko Town	Cockle	0.18±0.03^{h}	0.80±0.09^{g}	0.05±0.01^{g}	0.00±0.00^{d}
	Donax clam	0.94±0.02^{e}	1.75±0.07^{ef}	0.01±0.00^{g}	0.01±0.00^{d}
	Knife clam	0.51±0.08^{fg}	2.53±0.15^{c}	0.07±0.01^{g}	0.13±0.01^{d}
	Oyster	0.25±0.07^{h}	4.24±0.15^{a}	0.01±0.01^{g}	0.07±0.01^{d}

Means with different superscripts along the same column are significantly different (Duncan's test) $p<0.05$
Values are means ± standard deviation of triplicate samples

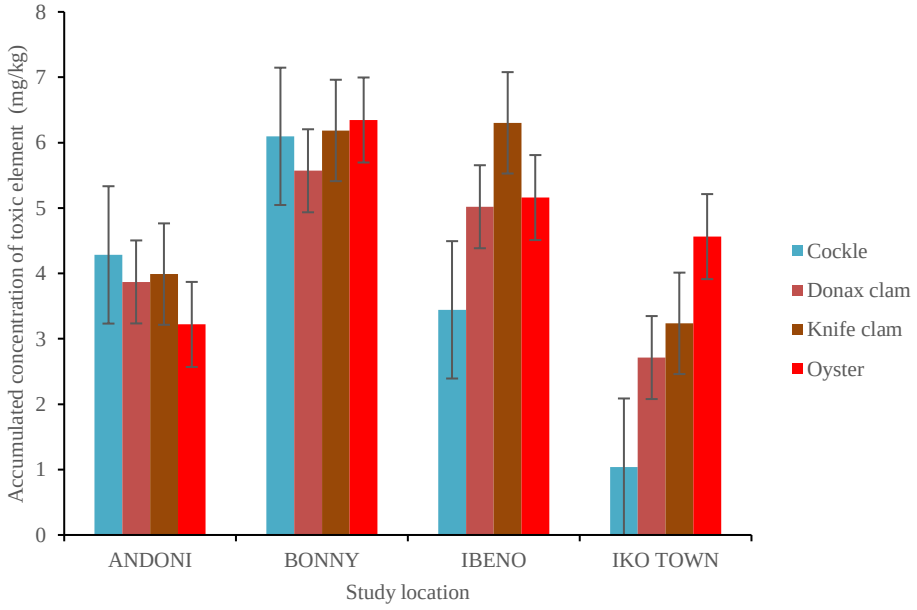

Figure 2. Accumulated toxic element concentrations in bivalve species from study locations

Bivalve molluscs have been extensively used as a model organism in environmental studies of water quality and biomonitoring agent hence the results from this study reflects the level of contamination of the Niger Delta environment as well as the safety of bivalve molluscs consumed in this area. According to **FAO (2003)**, and **EC (2008)**, regulations, lead concentration above acceptable limits 1.5mg/kg in bivalve tissue is unacceptable and can pose health risk to the consumers. Cadmium concentration above legal limits of 1.0 mg/kg and mercury above 0.5 mg/kg as well as the present of arsenic in the tissue of bivalve shellfish is dangerous to the health of the consumers. Therefore, the consumption of bivalve shellfish from the some of these locations would constitute a health risk because the concentrations of cadmium, arsenic and mercury above their safety limits. The concentration of toxic elements in the tissue of bivalve molluscs from this study were higher when compared to values reported by **Onwuteaka *et al.,* (2015)** at B/Dere in Ogoni land but lower than values reported by **Benson *et al.,* (2017)** at Qua Iboe estuary both in the Niger Delta region of Nigeria. The values are also lower when compared with the values reported by **Sarkar *et al.,* (2008)**, in bivalve shellfish at Sunderban mangrove Wetland at Bay of Bengal in India. Toxic elements are assimilated, stored, and concentrated by living organisms through food chain causing serious toxic effect to humans **(Giri and Singh, 2015)**. The toxicity of those elements is due to their ability to replace other metals in the active sites of enzymes, form complexes and precipitates with enzyme metals or other groups involve in metabolism, catalyzes the breakdown of essential metabolites as well as combined with membranes, thereby altering their permeability and hindering other elements in their electrochemical functions **(Burger and Gochfield, 2005, Oehlenschlager, 2002 and FAO, 2003)**.

For instance, lead and cadmium interact with essential elements such as zinc, iron, calcium and copper exerting an inhibitory effect on the activity of enzymes containing these mineral elements. This results in growth failure, improved nutrients tolerance, poor metabolism and absorption of these elements, reduction in plasma ceruloplasmic concentration among others **(Goyer and Clarkson, 2001 and Carvalho *et al*., 2005)**. Considering the study locations, samples from Bonny and Ibeno recorded higher accumulation of toxic elements (Fig. 2). This could be attributed the high population and industrial activities in these areas. Bonny hosts the Liquefied Natural Gas (LNG) Company, and Forcados terminal of Shell petroleum Development Company while Ibeno is a home to Mobile producing Nigeria Unlimited and other subsidiary companies. The discharged effluents alongside the activities such as artisanal refining of petroleum product and pipeline vandalization has negatively affected the environment resulting in elevated toxic element concentrations in seafood harvested from these locations raising serious concern about their safety for human consumption.

3.2 Non-Carcinogenic Risk of toxic elements

The estimated non-carcinogenic risk value or target hazard quotient (THQ) and hazard index (HI) are presented in Table 2 and Fig. 3. respectively. The non-carcinogenic risk values for lead indicated values ranging from 0.03 – 0.32. The values of THQ obtained for lead were less than the threshold limit of one. The non-carcinogenic risk values for cadmium ranged from 0.55-2.91, total arsenic ranged from 0.02-4.68 and methyl mercury ranged from 0.01-9.17. The non-carcinogenic values for total arsenic and methyl mercury at Iko Town were below the threshold limit of one while the non-carcinogenic values for cadmium, total arsenic and methyl mercury at Ibeno, Bonny and Andoni were above the threshold limit of one. Hazard index for toxic elements as shown in Fig. 3 were higher than the threshold limit of one except for bloody cockle harvested from Iko town. Also values for hazard index were highest in knife clam and mangrove oyster harvested from Bonny location.

The estimated non carcinogenic risk values were determined on the basis of the reference dose (RfD) for toxic elements as proposed by **US EPA (2009)**. According to **Saha and Zaman (2013)**, the RfD values represent the estimated daily exposure to which human population may continually be exposed over a life time without any appreciable health risk. The results for lead as obtained in this study is in agreement with reports of **Markmanuel and Horsfall (2015)**, **Archibong *et al* (2017)**, and **Udousoro *et al.,* (2018)**. According to **Uche *et al* (2017)**, whenever the non-carcinogenic risk value exceeded one, the estimated daily intake is relatively higher or more than the RfD. The estimated risk value for lead in this study is an indication that consumption of bivalve shellfish from the studied location would not likely increased health risk unrelated to cancer. However, excess consumption above 48g/day may likely result in eventual bioaccumulation and bioconcentration of lead which may result in serious deleterious health effects. The estimated non carcinogenic risk values for lead were less than the threshold limit of one indicating that consumers are unlikely to experience any significant health risk from lead through consumption of bivalve shellfish contaminated with lead in the Niger delta. The non-carcinogenic risk values for cadmium ranged from 0.55-2.91, total arsenic ranged from 0.02-4.68 and methyl mercury ranged from 0.01-9.17. The non-carcinogenic values for total arsenic and methyl mercury at Iko Town were below the threshold limit of one while the non-carcinogenic values for cadmium, total arsenic and methyl mercury at Ibeno, Bonny and Andoni were above the threshold limit of one which is similar to values reported by **Udousoro *et al.,* (2018)** in Periwinkle species from Ishiet and Ibeno coastal waters in Akwa Ibom State, Nigeria. Also, the non-carcinogenic values for methyl mercury and total arsenic were similar to values reported by **Archibong *et al* (2017)** on different fish species from Choba, Rivers state in the Niger Delta and **Lushenko (2010)** at the Imperial Beach, California. When the non-carcinogenic value is above the threshold value of one, there is likelihood of adverse health effects developing due to exposure to cadmium, total arsenic and methyl mercury at the oral ingestion of 48g/day of bivalve samples. According to **Khoshnood *et al*., (2014)**, the higher the non-carcinogenic risk value the higher the probability of risk on human body would be. Therefore, with the risk value of total arsenic and methyl mercury above 4 and 9 respectively is an indication that there is a greater risk for non-carcinogenic health effects for consumers of bivalve shellfish from the studied locations and this is a called for serious concern. A hazard index (HI) is the total chronic hazard attributable to exposure to all non-carcinogenic contaminants through the consumption of bivalve shellfish from the studied locations. It is calculated by summation of non-carcinogenic risk value for each species. There is no doubt that toxic elements such as Pb, Cd, As and Hg are present throughout the environment whether through natural or anthropogenic means and as long as the levels of these elements are continually increased due to pollution in the Niger Delta, and with the consumption of bivalve shellfish and other available exposure route in these coastal locations continuing unabated there is a likelihood of an adverse health effects considering values from the estimated non-carcinogenic values and hazard index calculated for toxic elements in this study. The higher levels of hazard indices for some element especially As and Hg could be attributed to various forms of anthropogenic activities such as environmental pollution occasioned by oil and gas exploitation, oil spillage and artisanal refining of petroleum product in these locations.

Table 2. Non-carcinogenic risk value of toxic elements in bivalves species

Location	Species	Lead (Pb)	Cadmium (Cd)	Total arsenic (As)	Methylmercury (MeHg)
Andoni	Bloody Cockle	0.27	1.00	1.94	4.21
	Donax clam	0.24	1.15	0.92	3.63
	Knife clam	0.12	1.40	0.28	8.18
	Mangrove oyster	0.20	1.01	1.87	3.98
Bonny	Bloody Cockle	0.29	1.70	3.23	4.50
	Donax clam	0.31	1.00	4.68	3.57
	Knife clam	0.19	1.54	3.73	9.10
	Mangrove oyster	0.32	1.22	3.56	9.17
Ibeno	Bloody Cockle	0.25	0.41	3.19	0.75
	Donax clam	0.19	1.78	1.90	4.07
	Knife clam	0.24	2.56	1.52	4.50
	Mangrove oyster	0.09	2.40	1.89	2.61
Iko Town	Bloody Cockle	0.03	0.55	0.11	0.01
	Donax clam	0.18	1.20	0.02	0.07
	Knife clam	0.10	1.73	0.17	0.89
	Mangrove oyster	0.05	2.91	0.02	0.46

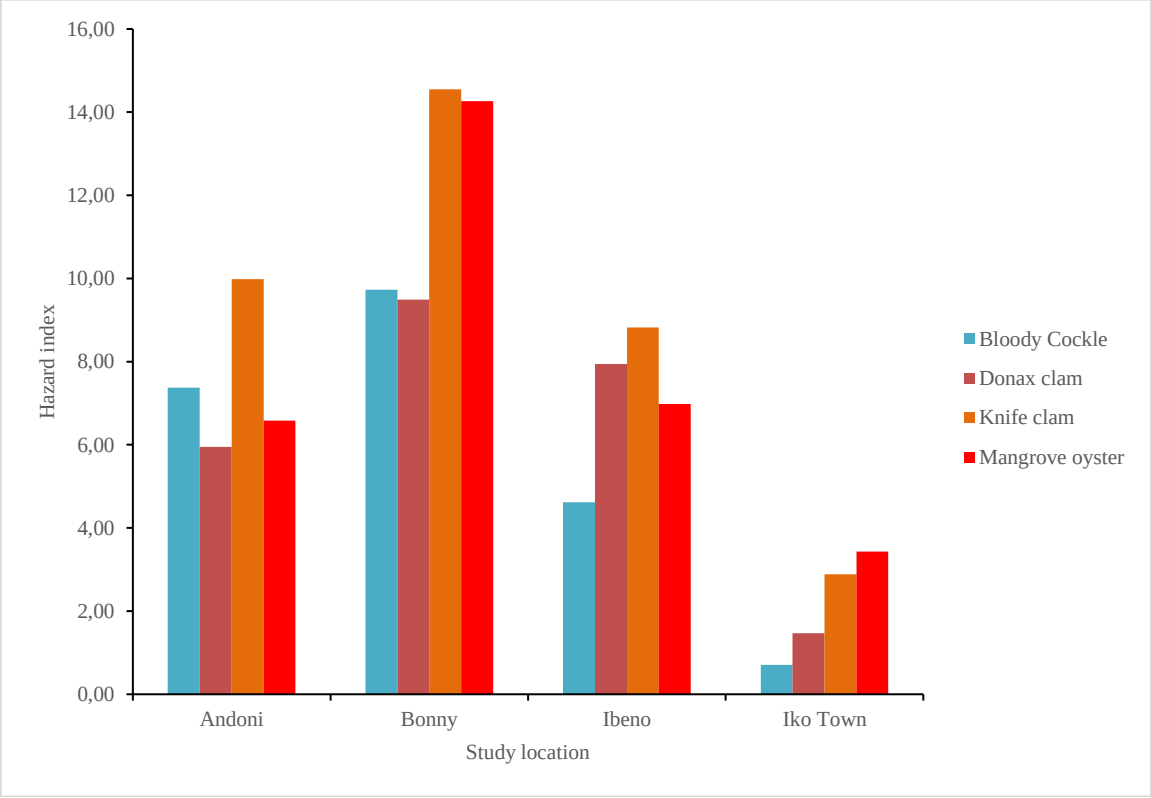

Figure 3. Hazard index of toxic elements in bivalve shellfish species

3.3 Carcinogenic Risk of toxic elements

The carcinogenic risk value or lifetime cancer risk for inorganic arsenic as presented in Table 3. indicated a cancer risk index higher than the acceptable limit of $1.0x10^{-6}$ in Andoni, Bonny and Ibeno when exposed to 48g/day of bivalve. Results showed that bivalve consumers from Bonny had the highest risk to the extent that 1 to 2 consumers in every 10,000 were likely to experience cancer related health conditions as a result of exposure to inorganic arsenic during their lifetime while consuming bivalve shellfish. Particularly, consumers from Andoni and Ibeno were also at a higher lifetime cancer risk ranging from 1 to 8 in every 100,000 people consuming bivalve shellfish due to their exposure to inorganic arsenic through consumption of contaminated bivalve shellfish. The carcinogenic risk index for bivalve consumers in Iko Town were within the expected limit.

Bonny, Andoni and Ibeno locations are host to multinational oil and gas industries which engaged in several forms of oil and gas explorations. These locations also experienced oil spillage and gas flaring which continually exposed these estuaries and coastal waters to various form of pollutions which may account for the results obtained in this study

Table 3. Carcinogenic risk value for toxic elements during bivalve consumption

Location	Species	Inorganic Arsenic	Risk Value
Andoni	Bloody Cockle	8.47E-02	8.71E-05
	Donax clam	4.03E-02	4.15E-05
	Knife clam	1.23E-02	1.27E-05
	Mangrove oyster	8.20E-02	8.43E-05
Bonny	Bloody Cockle	1.41E-01	1.45E-04
	Donax clam	2.05E-01	2.11E-04
	Knife clam	1.63E-01	1.68E-04
	Mangrove oyster	1.56E-01	1.60E-04
Ibeno	Bloody Cockle	1.40E-01	1.44E-04
	Donax clam	8.33E-02	8.57E-05
	Knife clam	6.63E-02	6.82E-05
	Mangrove oyster	8.27E-02	8.50E-05
Iko Town	Bloody Cockle	5.00E-03	5.14E-06
	Donax clam	1.00E-03	1.03E-06
	Knife clam	7.33E-03	7.54E-06
	Mangrove oyster	1.00E-03	1.03E-06

4. Conclusion

The present study confirms the occurrence and variability in the levels of toxic elements contaminants in the bivalve shellfish consumed by the coastal populations of the Niger Delta, Nigeria. Results provided qualitative information on the pollution status of lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) in the coastal waters of the study locations. Available assessments indicated that anthropogenic activities such as petrochemical operations, fuel combustion, oil spillage, and artisanal crude oil refining are very likely sources of toxic elements burden in the bivalve shellfish. Results from analysis of bivalve shellfish for toxic element concentrations and level of accumulation by bivalve shellfish revealed that bivalve shellfish are differentially selective for a range of toxic element and their concentration depends on the accumulation strategy adopted by each species for a particular element. Lead concentration was within the acceptable FAO limit of 1.5mg/kg while cadmium, arsenic and mercury were above their acceptable standards in shellfish which are likely to pose potential health risk to consumers. The estimated values for hazard indices and human health revealed a non-carcinogenic value and hazard index of less than one for lead while values for cadmium, total arsenic methyl mercury in Andoni, Bonny and Ibeno were higher than the threshold value of one indicating that consumers are not likely to experience any significant health risk through the consumption of 48g/day of bivalve samples. However, risk value for carcinogenic inorganic arsenic some study locations were higher than the stipulated one in one million ($1.0x10^{-6}$) chances which implies that carcinogenic effects were more likely due to consumption of 48g/day of bivalve shellfish with this contaminant. There is need for adequate strategies are to be adopted in order to control their presence of these toxic elements so that the possible health hazards to different life forms including man can be prevented also further studies on food safety risk assessment should be extended to more food matrices such as other benthic macrofauna, shrimps, crabs, catfish, sea snails and other important consumable seafood. This will help to generate enough evidence for regulatory and advisory purposes.

Declaration of interest

The authors report no conflicts of interest.

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Antifungal Activity of Topical Formulation Containing *Artemisia Herba Alba Asso* Essential Oil

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Abstract

Given the increase in antibiotic resistance phenomena and the undesirable effects of synthetic drugs. Medicinal plants are used as a direct or indirect source of the active ingredients. Our research constitutes a development of essential oils from *Artemisia herba alba Asso* cultivated in the Mascara region. A hydrophobic ointment has been formulated based on this essential oil and tested against five strains of *Candida albicans*. Several physicochemical and microbiological tests were used to verify the quality and the toxicity of the product. Then, the determination of the antifungal activity of this preparation was assessed against five strains of *Candida albicans* (S1, S2, S3, S4 and S5) by the disk-diffusion agar method. The results revealed that this preparation was devoid of total aerobic germs, yeasts and molds. However, the pH value was found equal to 5.98. In addition, the irritation primary index was marked less than 0.5. The ointment was powerful against S1, S3, S4 and S5 strains with inhibition diameters ranging from 16± 4 mm to 23 ± 2 mm. The Nystatin ointment was observed active against strains S2, S4 and S5 with diameters of the zones of inhibition; 21 ± 2 mm, 21± 1 mm and 20 ± 3 mm respectively. The ointment formulated with essential oil from *Artemisia herba alba asso* has proven useful against candidiasis caused by *Candida albicans* species.

1. Introduction

One of the major originalities of plants is their ability to accumulate frequently secondary metabolites, which represent an important source of molecules usable by humans in fields as different as pharmacology or the food industry **(Macheix *et al.*, 2005).** *Artemisia herba alba*, or known as sagebrush, is a plant characteristic of the Middle East, from North Africa, Southern Europe to the Himalayan mountains, it is used in herbal medicine to treat several diseases. Several structural types of sesquiterpene lactones have been found in the essential oils of the aerial parts of *A, herba alba*. The eudesmanolides followed by the germacranolides **(Bouldjadj, 2009).** The skin infection is usually treated with topical amphotericin, clotrimazole or nystatin **(Brown *et al.*, 2012).** The success of topical antifungal is attributed to its mode of action, the fungistatic doses and that they are not toxic on other host cells **(Martini, 2011).** Therefore, our choice fell on the formulation of a hydrophobic ointment based on the use of essential oils as an active ingredient. Then, its antifungal activity was carried out against five strains of *Candida albicans* by the diffusion method on agar.

2. Material and methods

2.1 Chemical products

Paraffin oil (Pharma services), glycerol (Pharma services), sodium benzoate (APAC), petroleum jelly (Phyto tech), dimethyl sulfoxide (Thermo Scientific), sterile tween 80 (Proteomic Grade), buffered sodium chloride-peptone (HIMEDIA).

2.2 Cultures

Cellulose filter membrane, 0.45 µm (Rotilabo), TSA medium (LABKEM), Sabouraud medium (Bio-Rad), nutritive broth (Bio-Rad), chloramphenicol-actidione medium (Bio-Rad), sterile physiological water, mueller Hinton agar (Bio-Rad).

2.3 Equipment

Mortar, pH meter (Radiometer), water bath (Memmert).

2.4 *Artemisia herba alba Asso* essential oil

Artemisia herba alba Asso was harvested in October 2015 in Mascara region and stored as voucher specimen in the herbarium of the university of Mascara under the code (AS00006). The extraction of essential oils and their compositions revealed by the GC / MS were described previously **(Boukhennoufa *et al.*, 2019).**

2.5 Animals

The rats belong to the *Rattus norvegicus* species were supplied by the pet store at the University of Mascara. However, the animals were acclimated to laboratory conditions at least five

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days before the experiment. The breeding was carried out according to the guide for the use and care of laboratory animals **(National Institutes of Health, 2011).**

2.6 Fungal strains

Five strains of *Candida albicans* were used for this study. The isolation and the identification of these microorganisms were described previously **(Boukhennoufa *et al*., 2019).**

2.7 Preparation of the ointment

The ointment is a hydrophobic type (Table 1) modified and adopted previously **(Biyiti *et al.,* 2012).** In a blender, the essential oil of *Artemisia herba alba asso*, paraffin oil and glycerol were introduced. The whole was brought to mixing for 10 min. Then, the sodium benzoate was added and mixed again for 10 min. The initial mixture recovered was placed in a mortar. The petroleum jelly was then added to the preceding mixture little by little by grinding until obtaining a creamy consistency and fine to the touch. The ointment was kept at 5°C in a dry place until use.

Table 1. Preparation of an ointment based on *Artemisia herba alba asso* essential oil (10%)

Constituants	Role	Quantity (g)
Artemisia herba alba asso essential oil	Active ingredient	10
Paraffin oil	Excipient	1.83
Glycerol	Excipient	10
Vaseline	Excipient	76.68
Tween 80	Emulsifier	0.49
Sodium benzoate	Conservative	1

2.8 Checking the quality of the ointment

2.8.1 Macroscopic characters
The macroscopic nature of the ointment was evaluated by observing the color, consistency and odor **(Sanogo *et al*., 2006).**

2.8.2 Homogeneity
The homogeneity of the ointments was checked by spreading a thin layer on a flat surface using a spatula. The regular distribution or not of the extracts in the excipients has been noted **(Sanogo *et al*., 2006).**

2.8.3 pH measurement
The pH value was determined by measuring a dilution to one tenth of each ointment in hot distilled water. Under the same conditions, the pH of each excipient was measured **(Sanogo *et al*., 2006).**

2.8.4 Microbiological quality
The ointments formulated have undergone microbiological quality control. The purpose of this test is to count total aerobic flora mesphile (TAFM), yeasts and molds. According to the monographs of the European Pharmacopoeia, the load of TAFM, yeasts and molds counted in a dispensing preparation will not exceed 100 CFU/g (**Pharmacopée européenne, 2002).**

2.9 Sample preparation

Ten grams of ointment was mixed with 2 g of sterile tween 80. Then, the mixture was introduced into a water bath set at 40°C. In order, to obtain a homogeneous mixture during handling. After homogenization, a volume of 90 ml of buffered sodium chloride-peptone (pH 7.0) was added to the preceding mixture. In order, to obtain a dilution of one in ten of the original product **(Pharmacopée européenne, 2002).**

2.10 Enumeration of germs

This protocol was applied according to the monographs of the European Pharmacopoeia (2002). The prepared sample (100 ml) was then passed through a cellulose filter membrane having a pore size of not more than 0.45 µm. The membranes were then transferred to the surface of the culture medium (TSA) for the enumeration of total viable microorganisms. While yeasts and molds were counted after the transfer of the membrane to the surface of the Sabouraud medium. Then, the incubation was done from 30 to 35 ° C for 5 days for bacteria and from 20 to 25°C for the demonstration of the presence of yeasts and molds **(Pharmacopée européenne, 2002).**

2.11 Determination of the primary irritation index (PII**)**

As part of the study of the antifungal activity of the prepared ointment, a determination of the primary irritation index was necessary in order to assess the degree of toxicity after short-term dermal exposure. This test was carried out **(Derelanko *et al*., 1993)**, where rabbits were substituted by rats. Because, of their availability and their physiology close to that of humans. The back of each animal was divided into two areas. One scarified and the other remains intact. Then, a weight of 0.5 g of the product was applied to the two parts and kept in contact with the skin by a dressing and an adhesive tape (Fig 1). Then the observations were made one hour, 24 and 72 hours after the application of the product. The PII value was calculated by an irritation and edema rating system. In order to bring out the average PI and to deduce the irritant effect of the product applied. The primary irritation index was determined by the following equation: **PI = (Edema + Erythema) Treated flank + (Edema + Erythema) Control flank / 24**.

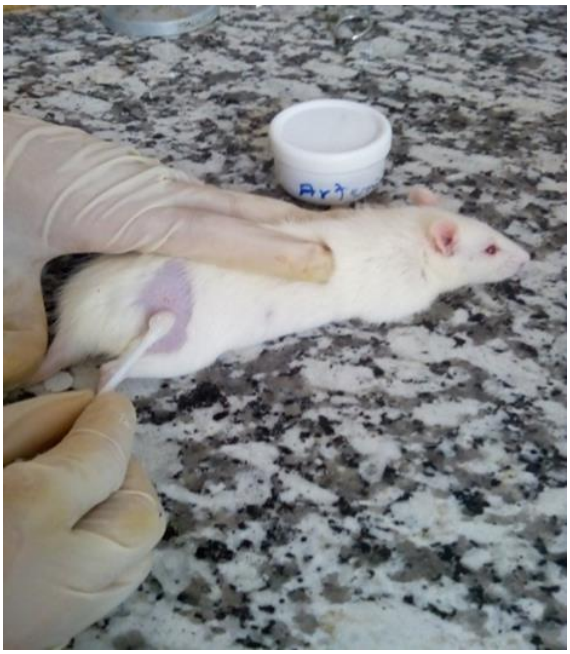

Figure 1. Ointment applying on the rat's skin to determine the primary irritation index

2.12 Antifungal activity of the ointment

The study of antifungal activity was carried out in a similar manner to that of an aromatogram. The reactivation was carried out in a liquid medium, nutritive broth for 24 h at 30°C **(Chopinet *et al.*, 2013**). Then a second subculture was carried out on Sabouraud-Chloramphenicol-actidione medium, incubated at 37 °C for 24 hours. From young cultures, three clones were inoculated into 5 ml of sterile physiological water. The suspension was standardized at the rate of 2 MC Farland **(Mighri *et al.*, 2010)**. 500 mg of the ointment were mixed with 1 ml of *dimethyl sulfoxide* (DMSO). At the same time, the Nystatin reference ointment was used as a positive control. However, the placebo was considered as a negative control. Petri dishes poured through the Mueller Hinton (MH) medium were inoculated beforehand with the strains (S1, S2, S3, S4 and S5) of *Candida albicans* by swabbing. The discs were impregnated with a volume of 10 µl of each solution and deposited on the surface. Then, the dishes were put at diffusion at 4 ° C for 30 min. Then, incubated for 48 h at 37°C. The effectiveness of the ointments against *Candida albicans* strains were expressed by measuring the diameter of inhibition all around the discs **(Ponce *et al.*, 2003).**

2.13 Statistical analysis of data

The experimental results were expressed as mean ± standard deviation (SD) of multiple replicates. Then, ANOVA (one way) was used to treat results, followed by Bonferroni's multiple comparison. The P values found below to 0.05 were considered statistically significant.

2.14 Ethics Statement

This study protocol was approved by the Local Ethical Comity of the University, based on adequately performed laboratory and animal experimentation according to the Helsinki Declaration (1964).

3. Results

3.1 Verification of the quality of the ointment

Drugs administered by the dermal route were much more effective and pose fewer problems than those of the other routes. This is why we choose the most classic formulation, the ointment. The quality of the ointment was assessed following numerous physical and microbiological tests (Fig 2). The formulated ointment was white in color, very smooth to the touch and easy to spread, with a characteristic smell of *Artemisia herba alba Asso.* The pH value was found equal to 5.98, this prevents the growth of germs promoting the degradation of its microbiological and organoleptic quality. On the other hand, no TAMF, yeasts and molds were detected on these products, in order to confirm their harmlessness before the determination of the antifungal activity *in vitro* against *Candida albicans.*

3.2 Determination of the primary irritation index (PII) of the formulated ointment

The PII determination results were determined using the Draize scores that appeared after 24 h and 72 h of application of the product. A value of 1 of erythema and edema of the sacrificial area were observed in the sixth rat after 24 h of skin contact with the product. These signs were completely gone after 24 h. While, the PII was found equal to 0.08. Since this value was strictly less than 0.5. Therefore, this ointment was qualified as non-irritating.

Figure 2. The formulated ointment

3.3 Study of the antifungal activity

A significant effect of the ointment was found against S1, S3, S4 and S5 strains with inhibition diameters ranging from 16 ± 4 mm to 23 ± 2 mm Compared to the placebo formulation with p value equal to 0.004. No significant effect of *Artemisia* ointment was observed compared to that of reference, nystatin against the tested strains (P= 0.66). However, the S2 strain was marked resistant to this ointment (Fig 3 and 4).

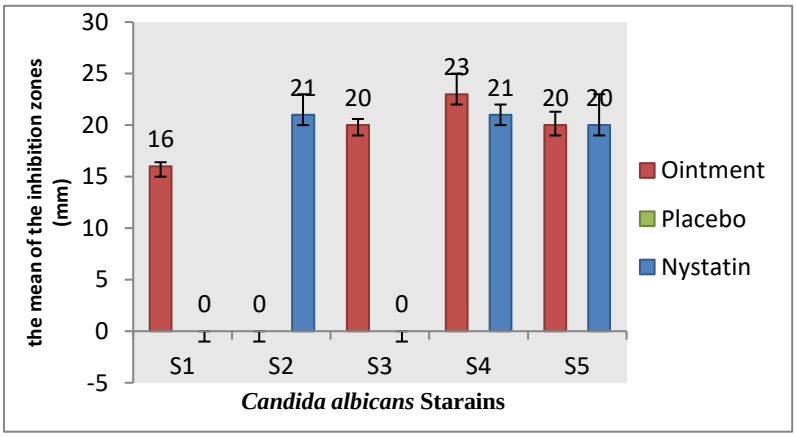

Figure 3. The average diameter of the inhibition zones, expressed in mm of the ointment, placebo and nystatin.

The negative control (placebo) did not induce any fungal inhibition. In addition, the reference ointment (Nystatin) was active against strains S2, S4 and S5 with diameters of the zones of inhibition: 21 ± 2 mm, 21± 1 mm and 20 ± 3 mm respectively. This difference in sensitivity against the fungal strains tested can be attributed to the quantity and quality of the active chemical compounds appreciated in essential oils.

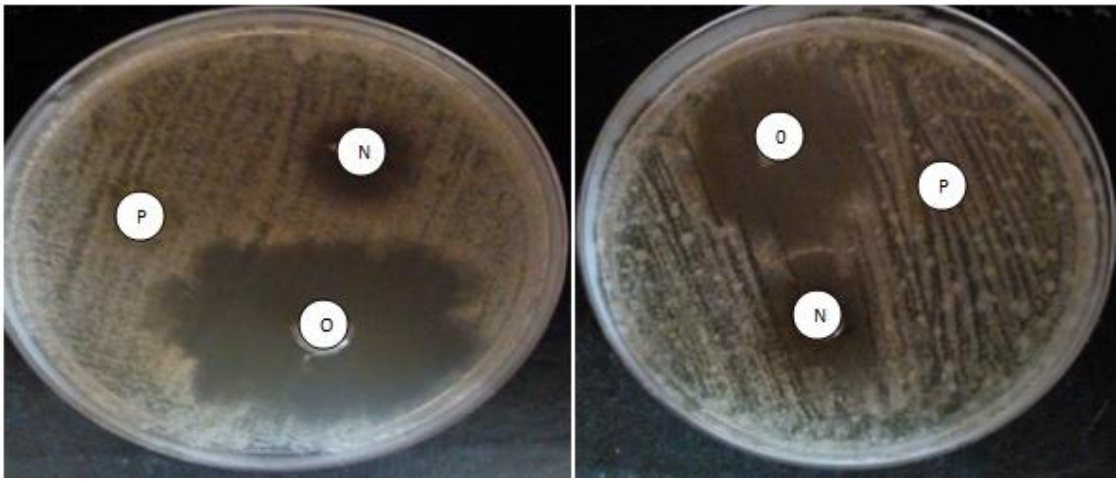

Figure 4. Inhibition zones of formulated ointment (O), placebo (P) and Nystatin (N). a: against the S3strain. b: against the S4 strain.

4. Discussion

The present study was conducted to determine the efficacy of an ointment contained *Artemisia herba alba asso* essential oil, used in the traditional pharmacopoeia of Mascara region. It should be noted that all the phenomena of erythema and edema were marked at the level of the scarified flanks. This is can be linked to the direct penetration of the ointment into the dermis. Because the epidermis layer is already damaged by scarifications. Any skin that is sick injured or modified by inflammation has an increased permeability of dermatological preparations compared to intact skin. And its risk must be taken into account **(Vandamme *et al.*, 2010).** By comparison with previous works, our results were similar to those reported, where she obtained a PII = 0.7 of an anti-cold ointment prepared with the essential oil of *Laurus nobilis* **(Guedouari, 2012).** The irritant power of a cream prepared from *Moringa oleifera* leaf extract was evaluated according to a single application by the 48-hour semi-occlusive patch test. However, no erythema or edema was observed after application **(Ali *et al.*, 2013).** In addition, the primary irritation index is equal to zero of an antibacterial soap prepared with oils from medicinal plants from the African pharmacopoeia, *Mareyami crantha, Mitracarpus scaberet Cassia alata* **(Soumahoro *et al.*, 2016).** In addition, the different effects of a single essential oil on the different fungal strains may be due to the variation in the genotype, although the five strains belong to the same species of *Candida albicans*. The antifungal activity of these essential oils can be attributed to its richness in oxygenated monoterpenes (80.85%) and in hydrocarbon monoterpenes (11.4%) already detailed previously **(Boukhennoufa *et al.*, 2019)**. The effect of the different doses of essential oil of *Artemisia herba alba asso* on the fungal strains, namely 0.05% and 0.25% indicate the mycelial decay of *Stemphylium solani*, *Fusarium moniliforme, Fusarium solani* and *Fusarium oxysporum* tested. While, the concentration of 0.75% completely prevented the mycelial growth of all the strains tested **(Goudjil, 2016)**. Camphor has been shown to be characterized by antibacterial, antidysenteric and fungicidal activity **(Tantaoui-Elaraki *et al.*, 1993).** In addition, hydrocarbon monoterpenes and oxygenated monoterpenes of essential oils were capable of destroying cellular integrity, resulting in inhibition of respiration and impaired permeability **(Cox *et al.*, 2000).** Indeed, α-pinene, β-pinene and limonene inhibit respiratory activity in the yeast mitochondria **(Tepe *et al.*, 2005).**

5. Conclusion

Faced with the concern of patients who often suffer from superficial and especially recurrent candidiasis, our study was considered as a first promotion of extracts from plants in order to develop new antifungal substances unknown by our organism, due to the increase in phenomenon antibiotic resistance. The ointment was found powerful against *Candida albicans* strains. However, this preparation was qualified as non-irritating on dermal route.

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Declaration of interest

The authors report no conflicts of interest.

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Bio-Fabrication, Spectroscopic Investigation and Antibacterial Potency of Ag-Co Bimetallic Nanoparticles Synthesized from the Root Extract of *Borassus aethiopum* (Palmyra Palm)

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Abstract

In this study, silver-cobalt bimetallic hybrid nanoparticles were synthesized using green method from $AgNO_3$ and $CoCl_2$ metal precursors as well as the locally available root extract of *Borassus aethiopum* acting as the reducing agent. The formation of bimetallic nanoparticles was first noticed by a color change of the reaction mixture from light pink to light brown as the result of Surface Plasmon absorptions. The optical measurements using UV-Vis showed the maximum absorption wavelength at 420nm while the functional group identification using FT-IR revealed some replacements in the absorption of functional groups, disappearance, and appearance of some others in the spectra of the BMNPs relative to that of the root extract indicating that those involved in the bio-reduction process. In vitro antibacterial potency was investigated against five clinically isolated bacteria. The outcome of the result suggested that they inhibit the tested bacteria especially against *Salmonella typhi*, *Bacillus subtilis* and *Klebsiella pneumoniae*. Thus, it can be developed as a bio-control agent for the treatment of diseases caused by these bacterial pathogens.

1. Introduction

Green is a term associated with a renewable product or non-toxic process whose introduction in the society has no harm or toxicity and a general favorable life cycle analysis and is non-persistant **(Lucia, 2016)**. Owing to the rich biodiversity of plants and their potential secondary constituents, plants and plant parts have gained attention in recent years as medium for nanoparticles' syntheses **(Roopan *et al.*, 2013)**. The main objective of the green chemistry is to decrease hazards associated with the processes and products that are important to the economy of the world and to maintain the good quality of life that we enjoy through chemistry **(Ismail *et al.*, 2018)**.

Recently, nanotechnology is developing and expanding and is used in many areas including health, nutrition, environmental health and agriculture **(Parang and Moghadamnia, 2018)**. Cobalt nanoparticles have catalytic, magnetic, optical, antibacterial and biomedical properties **(Igwe and Ekebo, 2018)**. High performance permanent magnetic properties and possess biomedical and cytotoxic activity **(Kuchekar *et al.*, 2018)**. Research has shown that nano sized silver particles have the ability to penetrate through the cell membrane, **(Chikkanna and Neelagund, 2018)**. Evidence suggests that germ cells and embryonic fibroblasts in rats are toxic with silver nanoparticles **(Parang and Moghadamnia, 2018)**.

Biosynthesis of bimetallic nanoparticles method is a good, low-cost and nontoxic method compared to physical and chemical methods which showed a high bioactive efficiency**, (Abd-Elsalam *et al.*, 2016)**. Bimetallic nanoparticles show better optical, electrical and medical applications due to their peculiar mixing patterns and synergistic effects of two metal nanoparticles that form bimetallic **(Mazhar *et al.*, 2017)**. In a study by **Parang and Moghadamnia (2018)**, Ag-Co NPs were synthesized using chemical reduction method and were found to have antifungal properties. **Cheng *et al.*, (2012)** reported the synthesis of Ag-Co bimetallic star shaped NPs and found out that they have the ability to be used in spectroscopy and catalytic applications. **Nelli *et al.*, (2002)** synthesized Ag-Co nanocomposite and studied the spectroscopic investigations using X-Ray spectrometry, TEM and energy loss spectroscopy.

Borassus aethiopum belongs to the family trees commonly known as Palm trees or simply Palms. It is a monotypic family in the order Arecales. The family contains several commercially important species such as coconuts, area nuts and date palms. Palms are well known for their great heights, exclusive foliages, conspicuous inflorescences and big seeds. Palms are predominantly perennial species and remaining green throughout the year **(Basu *et al.*, 2014)**. *Borassus aethiopum is* economically and medicinally useful. It is used as a vegetable, a beverage called arrack, crude sugar called jiggery. It has high transpiration rate thus has the potential to humidify the atmosphere and thereby having direct impact to cloud formation and rainfall **(Abdulrahman and Oladele, 2009)**.

This research is aimed at the green synthesis of Ag-Co bimetallic nanoparticles using aqueous root extract of *Borassus aethiopum*, its optical measurement using UV-Visible and functional groups

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determination using FT-IR spectrophotometers. Moreover, its bacterial potency was evaluated against five bacterial pathogens.

2. Material and methods

2.1 Plant Sample collection

Fresh roots of *Borassus aethiopum* were dug and collected from open area in Kaltungo Local Government Area, Gombe State and were brought to the Chemistry Laboratory, Gombe State University. They were identified by a botanist in the Botany Laboratory, Department of Biological Science, Gombe State University.

2.2 Sample preparation

Extract Preparation: Fresh roots of *Borassus aethiopum* were washed with tap water twice and then rinsed with de-ionized water so that contamination was minimized. They were then cut into smaller units and transferred into a crucible and were ground. A 30g of it was weighed and mixed with 200ml de-ionized water and warmed to 80°C for 30 minutes and was allowed to cool. The supernatant was filtered through Whatman number 1 filter paper and used immediately for the synthesis of the hybrid nanoparticles.

2.3 Synthesis of Silver-Cobalt Bimetallic Nanoparticles

A 100 ml of the prepared *Borassus aethiopum* root extract was mixed with 500ml of the mixture of the two metal precursors containing 250ml each of centimolar $AgNO_3$ and $CoCl_2$ (1:5 v/v) gradually while heating at 80°C for 30 minutes in a 600 ml beaker. Reduction of silver (I) and Cobalt (II) ion to their zero oxidation states were visually noticed by change in color from light pink to deep light brown. The solution was allowed to stay for 24 hours after which the nanoparticles settled at the bottom of the beaker. The supernatant was decanted and the residue was dried by evaporation.

2.4 Ultraviolet-Visible Spectrophotometer

The supernatant liquid was characterized using UV-Visible Spectrophotometer model 6705 for the wavelength range of 250 to 800 nm. Maximum absorption wavelength was determined by placing each aliquot sample in quartz cuvette operated at a resolution of 1 nm, using de-ionized water as the blank or reference solvent. The samples were placed in 1 x 1 cm quartz cell.

2.5 Fourier Transform Infrared Spectrophotometer

The synthesized silver-cobalt bimetallic nanoparticles and dried root extract samples were characterized using Fourier Transform Infrared Spectroscopy to determine the various functional groups involved in the bio-reduction process (PerkinElmer Spectrum Version 10.03.09 was used).

2.6 Determination of zone of inhibition using agar well diffusion method

Antibacterial activity of synthesized nanoparticles was assessed by the well plate agar diffusion method as described in the Aida modified method **(Aida *et al.*, 2001)**. The microbial cultures were adjusted to 0.5 McFarland turbidity standards; and inoculated on Mueller hinton agar plate of diameter 9 cm. The plate was flooded with each of the standardized test organism (1 mL), and then swirled. A sterile cork borer was used to make a 6 mm diameter wells on the agar plates. Aliquots of the nanoparticle dilutions were mixed with 50% DMSO at concentrations of 200µg/L, 300µg/L, 400µg/L and 500µg/L and applied on each of the well in the culture plates previously inoculated with the test organisms. Augmentin standard drug was used as the positive control for the bacterial studies. These were then left on the bench for 1 hour for proper diffusion of the nanoparticles. Thereafter, the plates were incubated at 37°C for 24 hours. Antimicrobial activity was determined by measuring the zone of inhibition around each well (taking the average of the length and breadth including that of the well) for each nanoparticles obtained from the plant extract.

2.7 Statistical Analysis

All data were analyzed using Microsoft Excel 2007.

3. Results and Discussion

3.1 Formation of the Ag-Co bimetallic nanoparticles

The formation of the bimetallic nanoparticles was first noticed by color change after addition of cobalt solution, Figure 1**A**, silver solution Figure 1**B** and Aqueous root extract of Palmyra palm, Figure 1**C** from milky, Figure 1**D** to light brown, Figure 1**E** within 15 minutes as a result of the surface Plasmon absorption. The Surface Plasmon absorption in the metal nanoparticles was due to the collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation. The supernatant was decanted and the solid nanoparticles settled at the bottom and further evaporated. Both the supernatant liquid and the liquid were used for further analysis. The change in color is shown in Figure 1 and 2 for the metal precursor and the synthesized bimetallic nanoparticles respectively.

3.2 Optical measurement using UV-Visible Spectrophotometer of Silver-Cobalt Bimetallic nanoparticles

The UV-Visible spectrum of the synthesized Ag-Co NPs is shown in Figure 2 below. The synthesis of bimetallic nanoparticles was followed by UV-Vis spectroscopy. The maximum absorption peak was shown at 420nm. The maximum wavelength is similar to that obtained by **Akinsiku *et al.*, (2018)**. The maximum absorption wavelength was greater than that observed for Cobalt nanoparticles (300nm) by **Igwe and Ekebo (2018)** and as well greater than that observed by **Chikkanna and Neelagund (2018)** for the synthesis of Silver nanoparticles. This suggests that bimetallic nanoparticles have higher wavelength compared to monometallic counterparts.

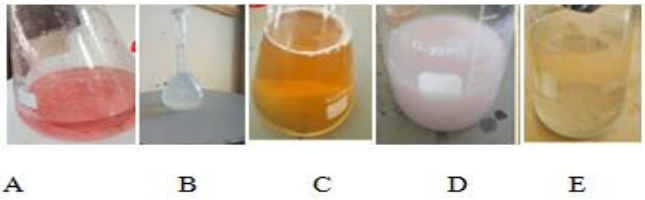

Figure 1. 0.01M $CoCl_2$ (**A**), 0.01M Ag (NO_3) (**B**), Aqueous root extract Palmyra palm(**C**), Mixture of Ag-Co immediately after addition(**D**), and Ag-Co BMNPs formation(**E**)

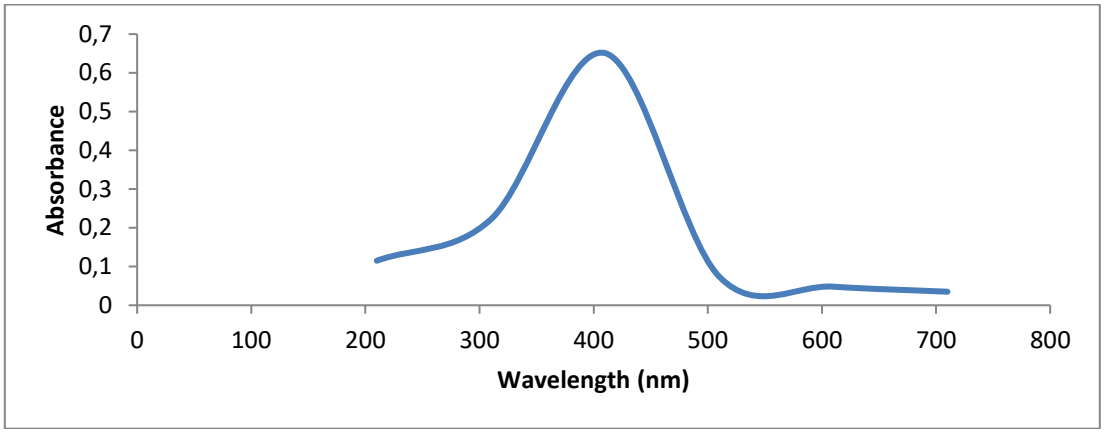

Figure 2. UV-Visible Spectrum for Ag-Co BMNPs.

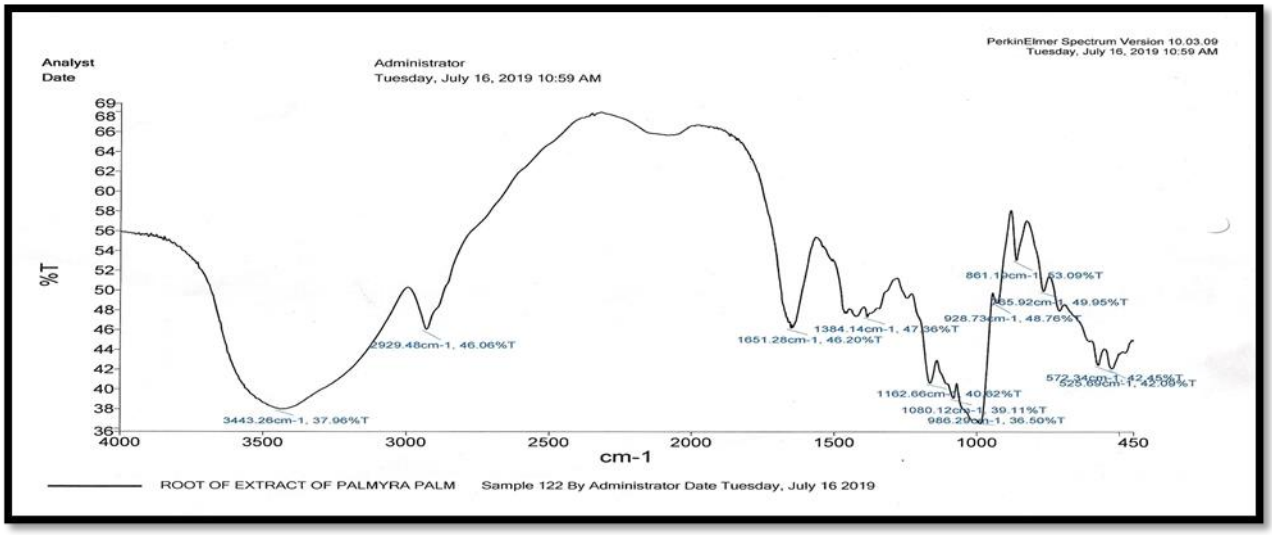

Figure 3. FT-IR Spectrum for root extract of *Borassus aethiopum*

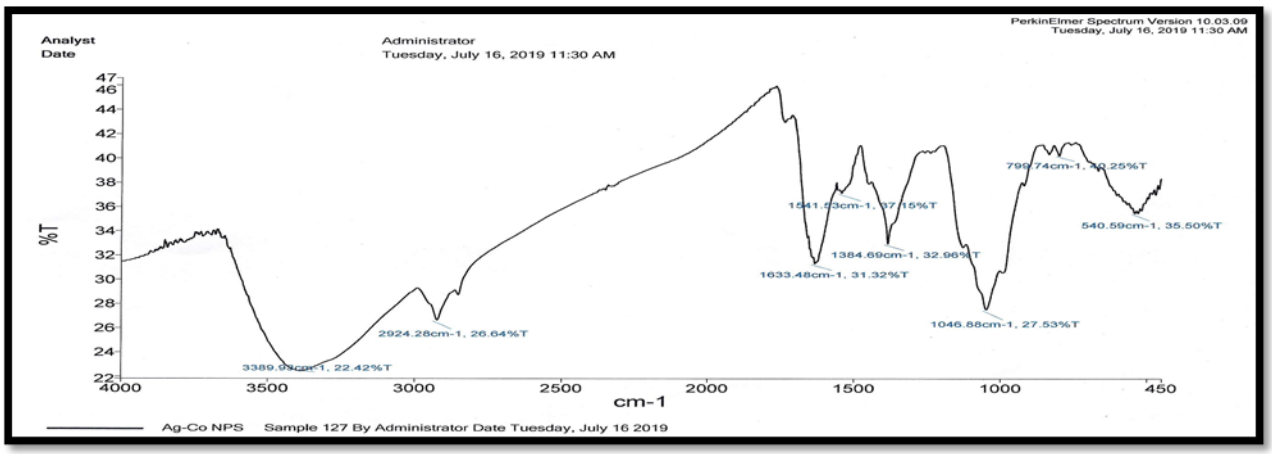

Figure 4. FT-IR Spectrum for Ag-Co BMNPs

3.3 FT-IR Result for Ag-Co BMNPs

FT-IR spectroscopy was applied to investigate the interactions between the aqueous root extract of *Borassus aethiopum* and the aqueous solution of the silver-Cobalt salt. The FT-IR spectra of the root extract and that of the biosynthesized Ag-Co BNPs are shown in Figures 3 and 4 respectively. Various functional groups present in the BNPS were identified. It displayed bands due to O-H stretching vibration mode at 3389.93 cm^{-1} which overlapped with the N-H stretching in terpenoid found within this region. Also observed, were a medium sharp peak for C-H absorption at 2924.28 cm^{-1}, C=C stretching at 1633.48 cm^{-1}, C=N stretching and the C-O deformation at 1541.53 cm^{-1} and 1046.88 cm^{-1} bands respectively. This is fairly the same with the result obtained by **Akinsiku *et al.* (2018)**. These have replaced those observed in the spectrum of the root extract that include peaks at 3443.26cm^{-1}, 2929.48 cm^{-1}, 1651.28 cm^{-1}, and 1080.12 cm^{-1}. Most notably is the appearance of a prominent peak at 1541.53cm^{-1} due to C=N stretching and the disappearance of the peaks at 1162.66 cm^{-1}, 986.29 cm^{-1}, 861.19 cm^{-1} and 525.69 cm^{-1}.

3.4 Antibacterial study results

Results of the antibacterial bioassay using agar diffusion test to identify the inhibitory activity of Ag-Co bimetallic nanoparticles against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis* and *Salmonella typhi* at different nanoparticle precursor concentrations are presented in Table 1 and a chart relating the zone of inhibition with concentration for the five bacteria is depicted in Figure 5.

The test showed the activity of the biosynthesized Ag-Co bimetallic nanoparticles was based on the size of zones of inhibition in millimeter (mm). Agar diffusion test revealed that the nanoparticles possessed antibacterial property due to the zones of inhibition values at a reasonable level. Interestingly, screened nanoparticles exhibited a dose-dependent inhibitory activity on the organisms in agreement with **(Andrighetti-Frohner *et al.*, 2009)**. It was also observed that the Ag-Co BMNPs displayed high activity on all the organisms considered even at low concentration of 200µg/L as compared with the standard drug Augmetin at concentration of 200µg/L, Table 1 and Figure 8. This showed that Ag-Co BMNPs have strong potency against the investigated pathogens. It is interesting to note that for all concentrations of Ag-Co BMNPs as well as standard Augmentin drug, equal zone of inhibitions was observed for *S. typhi* and *E. coli*, Table 1. Thus, Ag-Co BMNPs could be used in the treatment of these bacterial strain diseases. Previously, $CaO@SiO_2$ nanocomposite was synthesis by our research group and its antibacterial activity was tested on various pathogens **(Lamayi *et al.*, 2019)**. At 500 µg/L of $CaO@SiO_2$ nanocomposite, the inhibition zones were found to be 14, 16, 22 and 20 for *E.* coli, *B.subtilis* , *P.aeruginosa* ,and *K.pneumonia* respectively. These results are far lower than in the current study. However, at 500 µg/L of $CaO@SiO_2$ nanocomposite, the inhibition zone was found to be 26 mm for S. *typhi*. Therefore, for *E.* coli, *B.subtilis* , *P.aeruginosa* ,and *K.pneumonia*, Ag-Co BMNPs proved to be a promising antibacterial agent than *S. typhi* as compared to $CaO@SiO_2$ nanocomposite.

Table 1. Result for Antibacterial Studies of Silver-Cobalt Nanoparticles against 5-bacteria Pathogens

Zone of inhibition for Ag-Co BMNPs (mm)

Concentration of Ag-Co BMNPs (µg/L)	*E. coli* (mm)	*B.subtilis* (mm)	*P.aeruginosa* (mm)	*K.pneumonia* (mm)	*S. typhi* (mm)
200	13	15	17	9	13
300	15	20	18	14	15
400	21	17	25	15	21
500	22	30	23	28	22
Augmentin 300µg/L	11	8	23	8	11

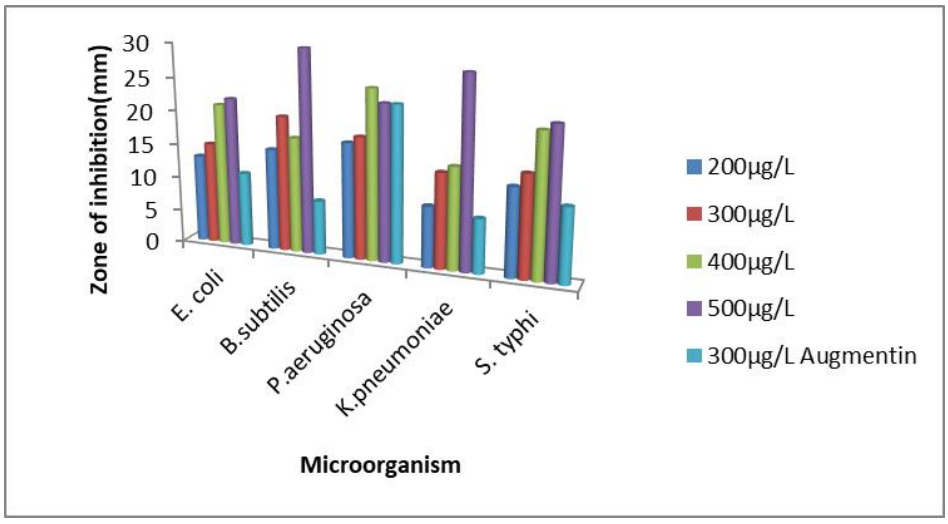

Figure 5. Chart for the antibacterial result for Ag-Co BMNPs

Conclusion

Motivated by the fact that little is known on the in vitro bacterial potency of Ag-Co bimetallic nanoparticles and other wider applications, these bimetallic nanoparticles were successfully synthesized from the locally available root extract of *Borassus aethiopum* and characterized using FT-IR and UV-Visible Spectrophotometers used frequently for identification of nanoparticles. Bacterial assay showed its effective efficacy as potential biocontrol for the treatment of bacterial diseases.

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Declaration of interest

Authors declare that there is no conflict of interest.

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Role of Environment, Nutrition, Microbiota, Mammalian Target of Rapamycin and Dietary Supplements in Autism

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Abstract

Autism Spectrum Disorder (ASD) is a developmental disorder with the age of onset under 3 years old. It is characterized by definite impairments in social interactions, speech abnormalities, and stereotyped patterns of behaviors. Although the exact pathology and etiology of ASD are not fully elucidated, exposure to environmental toxins, micronutrients deficiency, dysbiosis and mutation in genes of mammalian target of rapamycin (mTOR) signaling pathway are emerging as risk factors for ASD. Maternal exposure to heavy metals, air pollutants, and pesticides markedly increases the risk of ASD. Many clinical and experimental trials documented that gastrointestinal symptoms and disturbances of the gut microbiota usually accompanied cerebral disorders in autistic patients. Furthermore, studies showed that gene mutations causing hyperactivation of mTOR significantly lead to autistic symptoms. Pharmacological and nutritional interventions revealed a significant improvement in autistic individuals. The use of dietary supplements and the elimination diets exhibit minor or no adverse effects as compared to conventional drugs. In this review article, we tried to summarize some of the etiological factors that predispose to autism. We discussed the possible mechanisms that potentiate autistic symptoms by such factors. Also, we focused on the role of interventions either by various dietary supplements or by elimination diets in the management of autism.

1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition with age of onset under 3 years old. It is estimated that 1 in 160 children worldwide suffers from ASD. This estimate represents an average figure, and reported prevalence varies substantially across studies. The prevalence of ASD in many low- and middle-income countries is so far unknown (**WHO, 2018**). Based on epidemiological studies conducted over the past 50 years, the prevalence of ASD appears to be increasing globally. The disease affects males more than females (4:1), however, in girls the symptoms may be more intensified (**Rapin 1999; Solomon *et al.*, 2012**). ASD is characterized by a noticeable impairment of social interaction, delayed usage of language and behavioral disturbances such as self-injurious behavior (**Minshawi *et al.*, 2014**). Besides, the behavioral impairment, autistic patients has marked prevalence of gastrointestinal (GI) disease and dysbiosis (**White, 2003; Hsiao, 2014; Li *et al.*, 2017**), autoimmune disease (**Keil *et al.* 2010**) and mental retardation (**Noterdaeme and Wriedt, 2010**).

The etiology of autism is not clear, although various genetic, environmental, metabolic, neurologic and immunologic factors are probably involved. Environmental factors such as exposure to some toxic chemicals (heavy metals, pesticides, persistent and nonpersistent organic pollutants) can lead to neurological disorders (**Saghazadeh and Rezaei, 2017; Voorhees *et al.*, 2017; Guo *et al.*, 2018 b; Jeddi *et al.*, 2016**) respectively.

Early diagnosis and treatment for autistic patients exactly appear to improve their outcomes like a decreased need for special education and an increase in their independence (**Elder *et al.*, 2017**). Pharmacological, behavioral and nutritional interventions have been identified to minimize symptoms in autistic children (**Fung *et al.*, 2016; Cekici and Sanlier, 2019**). Meanwhile, the GI disturbances, intestinal mucosal abnormalities and altered intestinal microbiome (**Fulceri *et al.*, 2016; Li *et al.*, 2017**) extremely potentiate dietary intervention together with minimal or no side effects.

Adequate nutrition ensures normal central nervous system development and a diet rich in certain nutrients like omega 3 fatty acids keep mental function (**Lyall *et al.*, 2014**). Besides, adequate nutrients and nutrition have been documented to be essential in regulating molecular mechanisms that maintain synaptic function and plasticity **(Maynard and Mantini, 2017**). Moreover, cell proliferation, hormones and neurotransmitters' metabolism, and deoxyribonucleic acid (DNA) synthesis in the brain noticeably depend on sufficient nutrients (**Onaolapo and Onaolapo, 2018**). Previous studies have indicated that protein malnutrition and deficiencies of iron and iodine in early life predispose children to compromised growth and cognition (**Dosman *et al.*, 2007**). In this context, **Fujiwara *et al.* (2016)** found a strong correlation between malnutrition and autism.

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Ornoy *et al.* (2015) agreed with Fujiwara, they reported that obese mothers are liable to have autistic offsprings, On the other hand, specific maternal nutritional deficiencies are associated with increased ASD in their offsprings (**Surén *et al.*, 2013**). Additionally, experimental studies have demonstrated that propionic acid, a dietary short-chain fatty acid, induced a neuroinflammatory response and behavioral changes similar to those of ASD (**El-Ansary *et al.*, 2012**). Collectively, autistic children have higher food selectivity with a concomitant imbalance of nutrients levels in their blood (**Esteban-Figuerola *et al.*, 2019**) and the importance of dietary intervention is a must. So, our aim is to discuss nutritional and environmental factors involved in the occurrence of autism focusing on dietary intervention either by various dietary supplements or by elimination diets that may be effective for children with ASD.

2. Materials and Methods

PubMed database was used for the collection of data for the study in 2019. No limitation for the publication period was considered. Keywords were selected from the Medical Subject Headings and combined as "Autism Spectrum Disorder", "environmental factors", "nutritional factors", microbiota","mTOR", "dietary supplements" and "elimination diets".

Etiology of ASD

Environmental Factors

Recently, environmental factors have been involved in the etiology of ASD. There is an increasing growth in studies associating with environmental factors with ASD. Maternal environmental and or occupational exposures or both are non–genetic factors that may act during the prenatal period and cause neurodevelopmental deficits among which ASD has been introduced. Prenatal exposure to ambient air pollutants has been associated with the risk of ASD (**Gong *et al.*, 2017**). Residence in the proximity of areas with high levels of air pollutants emitted from industrial processes, biogenic sources, vehicular exhaust, and combustion products increases maternal risk of giving birth to an autistic child (**Weisskopf *et al.*, 2015**). It is obvious that exposure to air pollution and its components, both in the prenatal period and in early postnatal life, has been linked to poor developmental outcomes. **Kalkbrenner *et al.* (2014) and Karimi *et al.* (2017)** stated that some environmental exposures are linked with autism, especially traffic-related air pollutants, some metals, and several pesticides, with suggestive trends for some volatile organic compounds (e.g., methylene chloride, trichloroethylene, and styrene) and phthalates. They also concluded that other chemicals could not be ruled out. **Jeddi *et al.* (2016**) found a significant association between phthalate exposure and risk of ASD, whereas **Brown *et al*. (2018)** found no association between polychlorinated biphenyls (PCB) and risk of ASD.

Heavy metals are recognized as neurodevelopmental toxins which can lead to neurological defects, developmental delays, learning disabilities and behavioral abnormalities **(Gorini *et al.*, 2014). Mortazavi *et al.* (2016)** introduced the hypothesis that maternal exposure to electromagnetic fields increases the release of mercury from dental amalgam fillings. They suggested that such a rise in the level of mercury may be a possible mechanism for high rates of autism in offsprings. Blood levels of mercury, arsenic, cadmium, and lead has been investigated in one hundred eighty unrelated children with ASD and 184 healthy controls (**Li *et al.*, 2018**). Data showed that the children with ASD had significantly ($p < 0.001$) higher levels of mercury and arsenic and a lower level of cadmium. The levels of lead did not differ significantly between the groups. The results of this study are consistent with several previous studies, supporting an important role for heavy metal exposure, particularly mercury, in the etiology of ASD (**Talbott *et al.*, 2015; McCaulley, 2019**). This could be explained by the possibility that women with chronic metal exposure accumulate high tissue levels of mercury and other metals. Consequently, they may pass potentially toxic metals to their fetuses or intoxicate infants during nursing. Molecular mechanisms by which metals trigger neurobehavioral disorders and, specifically, ASDs are still not completely clear. In general, some environmental factors or perinatal complications might cause a pro-inflammatory state and oxidative damage in the brain and subsequently lead to alterations in neural growth and development (**Goines and Ashwood, 2013**). High body burden of toxic metals in autistic patients is associated with oxidative stress and increased levels of the ratio of glutathione disulfide (GSSG) to glutathione (GSH) (**Adams *et al.*, 2011**). Glutathione-S-transferase (GST) genes and enzymes play a major role in detoxification of several heavy metals. A recent systematic review suggested gender-related differences in the susceptibility to metals, with boys generally more susceptible than girls (**Llop *et al.*, 2013**). Metals such as lead, mercury, and cadmium have the ability to interfere with physiological thyroid hormone levels (**Chen *et al.*, 2013**). The interaction of metals with hormones and neurotransmitters may represent one of the neurotoxicity mechanisms involved in ASDs (**Hall and Kelley, 2014**). **Kinney *et al.* (2010)** reported that some environmental factors such as certain toxins and vitamin D deficiency increase the risk of a gene mutation that in turn can lead to an increased risk of ASD. Moreover, other environmental factors interact directly with neurotransmitter pathways. For example, lead disrupts the activity of N-methyl-D-aspartate receptors (**Neal *et al.*, 2010**).

More work is mandatory to estimate the effect of various chemicals and xenobiotics on the occurrence of ASD. Exposure to harmful environmental factors can alter the expression of developmental key genes in critical periods of embryo formation and raises the potentiality of genomic imprinting diseases such as autism (**Karimi *et al.*, 2017**). None of the environmental factors alone are sufficient to induce autism, but rather a collection of them can be involved in the incidence of autism (**Kim and Leventhal, 2015**).

Nutritional Factors

The role of nutrition in ASD etiology begins as early as the prenatal period. Maternal nutrients deficiency has been strongly associated with increased risk of schizophrenia, neural tube defects and many neurodevelopment disorders (**Furuse *et al.*, 2017**). Nutritional deficiencies are particularly increased during gestation due to elevated metabolic needs imposed by the fetus, placenta, and maternal tissues and have proven to influence fetal brain development in terms of function and structure. Therefore, a pregnant woman's diet could affect brain development which may increase autism risk. However, relatively few studies have been dedicated to understanding how maternal dietary factors could influence offspring brain development. **Schmidt *et al.* (2011)** suggested strong protection of prenatal high levels of folic acid from autism. Moreover, the risk of autistic disorder was 40% lower among those whose mothers administered folic acid supplements, 6 weeks before and after conception (**Surén *et al.*, 2013**). Also, it was proven that pregnant women who are obese or suffering from diabetes may give birth to an autistic child (**Connolly *et al.*, 2016**).

Maternal fish intake may also be important for ASD, as a source of essential fatty acids and vitamin D. Children of mothers with increased intake of omega 3 fatty acids before and during pregnancy had reduced risk of ASD relative to children of

mothers of low intakes of omega 3 fatty acids (**Lyall *et al.*, 2014**). In contrast, **Lyall *et al.* (2013)** found no association between prenatal fish oil and ASD.

Low maternal and fetal vitamin D levels have been proven as a risk for ASD (**Ali *et al.*, 2019**). Vitamin D insufficiency in mothers has been linked to impaired language skills in their children at ages 5-10 years (**Whitehouse *et al.*, 2013**). Vitamin D influences neuronal differentiation, metabolism of neurotrophic factors and neurotoxins, protection from brain inflammation, endocrine function and fetal brain growth. Also, vitamin D can decrease the risk of viral infection for pregnant woman. Infectious disease during pregnancy adversely influences brain development that may lead to autism. Vitamin D level is important, especially in the third trimester of pregnancy when the fetal brain develops (**Larqué *et al.*, 2018**). Thus, vitamin D supplementation during pregnancy can confer protection against autism either directly or indirectly by aiding proper brain development and enhancing the immune system, respectively. Moreover, during the perinatal period, maternal diet plays a crucial role in the maturation of the vital organs and neuronal connections. If nutrition is deficient of specific micro or macronutrients or overloaded with excess calories, many developmental disorders can be devastating and long-acting because the brain is especially sensitive to prenatal nutrition. Autism is hypothesized to be attributed in part to such factors that may date back to very early life (**Moody *et al.*, 2017**).

The gut microbiota

The human gut contains up to 100 trillion micro-organisms including different species of bacteria. Bacteroidetes and Firmicutes phyla are the most predominant bacterial species in the human microbiota (**Eckburg *et al.*, 2005**). Although the exact etiology and pathology of autism still not obvious, gut-brain interactions have received certain attention. There is a complex bidirectional axis between the gut microbiota and the brain (**Liu *et al.*, 2019**). Communication along this axis principally shows how signals from gut microbiota affect brain function, and on the other hand, brain messages influence microbiota activity and other GI functions. This bidirectional communication is mainly achieved through neuroendocrine and neuroimmune mechanisms (**Mayer, 2011**). In autistic patients characteristic neurological deficits are generally associated with various GI symptoms (**Adams *et al.*, 2011**). It was documented that in ASD, the intestinal inflammation is often accompanied by elevated neuroinflammatory markers and reduced serotonin levels in the brain (**de Theije *et al.*, 2014**). Such deficits and symptoms may be initiated from dysbiosis or microbial imbalance which could disturb the coordination of the gut microbiota-bran axis (**Pulikkan *et al.*, 2019**). Metagenomic analysis proved that autistics have a decrease in Bacteroidetes, an elevation in the ratio of Firmicutes to Bacteroidetes, and an increase in Betaproteobacteria (**Parracho *et al.*, 2005**). Moreover, Clostridia, Bacteroidetes, and Desulfovibrio are common bacteria that may promote GI symptoms and neurological autistic behaviors (**MacFabe, 2012**). They not only modulate the intestinal immune system but also produce certain metabolites that contribute directly to autism pathology. For instance, Colistridium produces a potent neurotoxin that manifests a wide variety of behavioral deficits seen in autism (**Bolte, 1998**). Besides, it's another metabolite,3-hydroxyphenyl-3-hydroxy propionic acid (HPHPA) depletes catecholamines in the brain, consequently, genus Clostridium is strongly correlated with the etiology of autism (**Kesli *et al.*, 2014**).

Generally, the colonization of gut microbiota commences at the time of birth on exposure to vaginal microbiota. The host genomic significantly influences microbiota activity and diversity. Also, environmental factors that include, infections, diet, stress, diseases and antibiotics may alter microbiota natural composition (**Nicholson *et al.* 2012**). Additionally, adult maternal immune activation results in fetuses that have ASD features, dysbiosis in gut microbiota and an altered blood metabolic profile (**Hsiao *et al.*, 2013**). Besides, recent studies have documented that probiotics and prebiotics administration may be an effective therapy for autistics via modulation gut microbiota (**Parracho *et al.*, 2010**; **Liu *et al.*, 2016**) respectively.

The mammalian (mechanistic) target of rapamycin (mTOR)

ASD exhibited aberrant expression of various genes, but those involved in the mTOR signaling pathway like Neurofibromatosis type 1 (NF1), Tuberous sclerosis proteins 1 and 2 (TSC1, TSC2), Phosphatase and Tensin homologue (PTEN), and Fragile X mental retardation protein (FMRP) are the most associated with autism. Single gene mutations result in enhanced mTOR activity in the brain of the ASD model (**Ehninger and Silva, 2009**). Consequently, an increase in the phosphorylation of proteins is observed with concomitant elevation of neuroligins that participate in the formation and maintenance of synapses between neurons. This caused an increased synaptic excitation/inhibition ratio which may be a risk for ASD (**Wang and Doering, 2013**). Additionally, the mTOR-signaling pathway has a potential role in directing the immune responses. **Kim *et al.* (2008)** proved that mTOR complex1 (mTORC1) when activated in mast cells results in survival, differentiation and cytokine production. Moreover, mTOR activation is shown to markedly attenuate autophagy (**Yu *et al.*, 2010**) in the intestine that is essential to limit intestinal inflammation. Therefore, a loss of both immune regulation and intestinal barrier integrity resulted from mTOR hyperactivity, thus, in ASD distribution of the mTOR signaling pathway could disrupt immune response, gastrointestinal tract, and brain. By this way, mTOR signaling in ASD may be considered as an important factor in the gut-immune-brain axis (**Van Sadelhoff *et al.*, 2019**). Therapeutic strategies for autism could manage the signaling pathway. **Kotajima-Murakami *et al.* (2019)** documented that pharmacological treatment by rapamycin successfully recovered mutations in the expression of some mTOR genes with consequence improvement in social communication in the ASD model. However, intervention with some nutrients like amino acid may manipulate the mTOR signaling pathway with minor or no adverse effects. They are capable to inhibit mTOR, inflammation, improve gut barrier function and normalize microbiota composition and immunity in the ASD patients (**Van Sadelhoff *et al.*, 2019**). Interestingly, cellular levels of different amino acids are maintained through the mTOR signaling pathway and the disturbance of this pathway significantly dysregulates amino acids that is strongly associated with autism (**Maynard and Manzini, 2017; Smith *et al.*, 2019**)

Nutritional assessment of autistic patients

Several studies have high lightened that individuals with autism are nutritionally vulnerable because they have a picky eating pattern and sensory sensitivity; both predispose them to restricted dietary intakes (**Emond et al., 2010**). Nutritional assessment includes dietary, anthropometric and biochemical evaluations for autistic patients. In addition, clinical examination that assesses the patients for signs which are consistent with nutrient deficiencies. Finally, the environmental factors assessments that affect the nutritional status, such as socioeconomic status and lifestyle (**Ranjan and Nasser, 2015**).

Dietary measurements

They include both qualitative and quantitative assessments of dietary intake which could determine adequacies and inadequacies inpatient nutrient intake. Consequently, nutritional status can be evaluated. The majority of autistic children exhibited nutritional challenges that include difficulty accepting new foods, late acceptance of solid food, restricted intake of food based on its color, texture, appearance etc, meal time presentations difficulties like position of food on a plate or even the type of plate, increased sensory sensitivity, disruptive mealtime behaviors such as not eating with family, eating the same foods and refusing the change and finally, the pica behavior. Autistic children are called picky eaters; however, this habit does not correlate with a lack of appetite (**Williams *et al.*, 2000**). They more frequently accept food of low texture and highly energetic and particularly refuse vegetables. Although they eat less variety of foods, their total calories, carbohydrates or fat intakes are not statistically different as compared with normally developing children (**Emond *et al.*, 2010**) indicating that their satiety mechanisms are not impaired. Their protein intake is approximately similar to normal children, but the nondairy protein intake was increased in ASDs children (**Zimmer *et al.* 2012**). Therefore, it is obviously clear that macronutrient deficiencies are often not present in children with autism (**Herndon *et al.,* 2009**). Besides, a substantial number of autistic children had inadequate intakes of calcium, zinc, iron, and vitamins; D, C, E, riboflavin, B12, and folic acid and choline, which may be attributed to less consumption of vegetables, fruits and salads with concomitant reduction of these essential micronutrients (**Bandini *et al.,* 2010**). In the contrary vitamin B6 intake was found to be significantly higher in autistic children than typically developing children. However, deficiencies of micronutrients in autistic children could be overcome by giving those fortified foods rather than additional vitamins or food supplements.

Anthropometric measurements

Anthropometric assessments include height, body mass index (BMI) and head circumference (HC). Early recognition of such measurements will serve as a noninvasive, inexpensive, and objective method of nutritional status evaluation. Such measurements have been carried out with autistic children and compared them with typically developing controls. An early warning signal of vulnerability to autism may be an abnormally accelerated rate of growth (**Courchesne *et al.,* 2001**). This growth abnormality may be attributed to nonspecific expression of biological abnormalities that are present in these disorders. There are inconsistent results in the prevalence of obesity in autistic children. Some studies reported an overweight or obesity prevalence in ASD patients, which was similar to nonautistic children (**Curtin *et al.,* 2010**). Other studies found a higher prevalence of obesity in autistic children than controls (**Egan *et al.,* 2013**) which may be explained by their unusual dietary patterns that are accompanied by decreased access to appropriate physical activity. Concerning HC, studies showed that autistic children have an atypical head growth pattern that is at birth, they have a normal HC, followed by an increase in the rate of growth of HC until one year of age. Then, there is a rapid decrease in HC between 12 and 24 months, which is normal as compared with controls (**Redcay and Courchesne, 2006**). Many authors have postulated that this abnormally accelerated growth in HC is attributed to dysregulation of growth in general. The relation between HC and height is inconsistent. Some studies stated that HC is relatively increased in comparison with height (**Grandgeorge *et al.,* 2013**), others reported that HC is normal (**Mraz *et al.* 2007**) or smaller (**Schrieken *et al.* 2013**) relative to height.

Biochemical measurements

Estimations of nutrients and nutrient-related substances in biological specimens are important in the diagnosis of autism before the clinical signs or symptoms are apparent. Besides, the obtained knowledge of this analysis helps to determine the treatment plan and monitor its effectiveness. Autistic children have decreased concentrations nearly below the reference range of folate and vitamin B_{12} (**Ali *et al.,* 2011**), pantothenic acid, biotin and vitamin E (**Adams *et al.,* 2011**) and vitamin D (**Meguid *et al.* 2010**). Meanwhile, vitamin B6 has an elevated and broad distribution in autistic children that is very fascinating. The highest concentration of vitamin B6 may be explained by a low activity of pyridoxal kinase that converts pyridoxal and pyridoxine into the active form pyridoxal 5-phosphate (PSP). Compared with controls, autistic children have lower concentrations of lithium, calcium, magnesium (**Adams *et al.*, 2011**), iodine and chromium (**Adams *et al.,* 2006**) and selenium (**Lakshmi Priya and Geetha, 2011**). The significant correlation between vitamin D (25-hydroxyvitamin D) and calcium supports the idea that autism is a vitamin D deficiency disease (**Meguid *et al.*, 2010**). By contrast, copper, phosphorus, and boron were elevated in autistic children (**Adams *et al.*, 2011; Ranjan and Nasser, 2015**) and also mercury and lead. On the other hand, the prevalence of iron deficiency and anemia in ASD patients is reported (**Bilgiç *et al.*, 2010**). However, iron deficiency results in impaired cognition and developmental defects which could further compromise their behavior and communication. Concerning amino and fatty acids, autistic children have lower levels of plasma tyrosine and tryptophan (**Adams *et al.*, 2011**) which may impair serotonin synthesis that has an important role in the neurogenesis and also in neurotransmission. The contributory factors to such decreased amino acid levels may be decreased protein intake or digestion by autistic children. Autistic patients have elevated glutamate levels in plasma (**Aldred *et al.*, 2003**) that may be closely related to the behavioral changes in autism. Moreover, omega 3 polyunsaturated fatty acid concentration is significantly lower in children with autism (**El-Ansary *et al.*, 2011**).

Dietary interventions

Dietary intervention mostly includes either: Dietary supplements or Elimination diets or both together (**Adams *et al.*, 2018; Fraguas *et al.*,2019**). Successful dietary interventions could quickly relieve the autistic symptoms and are usually used as complementary with conventional pharmacological drugs.

Dietary supplements

Folic acid and vitaminB_{12} supplements

Both folic acid and B_{12} participate in the methionine cycle that involves the regeneration of methionine through the transfer of the methyl group from 5-methyltetrahydrofolate.
Methionine forms S- adenosylmethionine (SAM) which is the primary methyl donor for DNA, Ribonucleic acid (RNA), phospholipids, proteins, and neurotransmitters. Another important role for the methionine cycle is the production of glutathione, a crucial antioxidant compound. Vitamin B_{12} and folic acid deficiencies were seen in many autistic children (**Ali *et al.* 2011**). Moreover, patients with autism exhibited cerebrospinal fluid (CSF) deficiency of folic acid that may be attributed to the action of serum antibodies against foliate receptors. These antibodies bind folic acid receptors with

concomitant inhibition of folic acid synthesis and reduction level in CSF (**Ramaekers *et al.,* 2005)**. In this view, **Mckee *et al.* (2017)** pointed out that dietary methyl donor supplementation in early life can change cognitive performance and motivation. Meanwhile, vitamin B_{12} deficiency may result from a digestive cause, especially rare consumption of animal sources. **Pineles *et al.*** (2010) demonstrated reversible optic nerve neuropathy in autistic patients via vitamin B_{12} replenishment. Hence early detection of folic acid and vitamin B_{12} deficiencies may be an essential contributory factor in preventing ASD or in determining the therapeutic interventions for autistic ones. Also, a diet rich in these nutrients or supplements may be supportive of pharmacotherapy.

Vitamin C supplement

Vitamin C is essential for the synthesis of neurotransmitters and via its antioxidant power, it protects the brain and nervous tissue against free radicals. **Planerova *et al***. (2017) demonstrated that ASD patients may have scurvy, which results from vitamin C deficiency which was a consequence of typical food consumption by those patients. **Malhi *et al.*** (2017) agreed with the previous authors in that ASD children failed to achieve vitamin C requirements. Moreover, moderate doses of vitamin C with other vitamins may affect sleep disorders and gastrointestinal troubles in ASD patients (**Adams and Holloway, 2004**). Besides, vitamin C supplementation in an autistic patient with normal or low serum vitamin C level, may exhibit a positive impact concerning the pathological behavior through prevention of dysregulation of glutamatergic signaling of the brain, consequently, reducing brain inflammation (**Blaylock and Strunecka, 2009**). Vitamin C could be complementary to conventional therapy taking into consideration its tolerance in autistic patients, therefore, continuous monitoring is essential.

Vitamin B_6 supplement

Dietary vitamin B_6 supplements were proven to improve behavior in autistic children (**Martineau *et al.*** 1985). Meanwhile, **Wong and Smith (2006)** demonstrated that no marked benefits of vitamin B_6 administration in ASD children. However, vitamin B_6 has an essential role in CNS as it participates in neurotransmitter synthesis like serotonin, dopamine, and epinephrine which may be altered in autistic children. Therefore, vitamin B_6 supplementation could be beneficial for autistic patients, taking into consideration to adjust its doses because high blood levels are accompanied by low activities of both kinase and oxidase enzymes that transform vitamin B_6 to the active form PSP.

Vitamin A and D supplements

Many autistic patients have vision problems besides other behavioral and clinical symptoms. Hence, the administration of vitamin A may be helpful (**Uyanik, 2006**). In agreement with the previous author, **Megson (2000)** demonstrated that vitamin A supplement is effective in reducing autistic symptoms since there was an absence of specific genes in ASD patients encoding an essential protein for vitamin A synthesis. Moreover, **Guo *et al.* (2018 a**) proposed that vitamin A supplementation may improve symptoms and reduce 5-hydroxytryptamine(5-HT) levels in autistic children. Hence, vitamin A supplementation is a rational therapy for children with autism. Concerning vitamin D, it was proven that adequate intake of this vitamin from a diet or as a supplement may reduce the risk of autism by providing the proper development of the brain and immune system. It also has a neuroprotective effect and influence many neurotransmitter interactions (**Meguid *et al.*, 2010**). **Jia *et al.* (2015**) concluded that vitamin D3 may play a significant role in the etiology of ASD. In this context, **Feng *et al.* (2017)** reported that autistic children exhibit clinical improvement after vitamin D_3 supplementation. While **Kerley *et al.* (2017)** reported that vitamin D supplementation did not affect the primary outcome with limited and incompatible effects in children with ASD. This may be attributed to vitamin D activity and dose.

Probiotics supplement

Autistic patients frequently suffered from several gastrointestinal disturbances like diarrhea, constipation, and inflammation. Therefore, the use of probiotics may be beneficial as they help to restore the normal intestinal microflora and normal intestinal epithelial cells, consequently reducing gastrointestinal disturbances. Moreover, probiotics could increase the utilization of food ingredients and vitamin synthesis by the body that may be helpful for autistic patients who have multiple nutrient deficiencies. Besides, probiotics enhance immunity and inhibit many pathogens developments (**Galdeano *et al.*, 2019**). Probiotics are also supposed to improve intestinal permeability, enhance the attainment of a balanced intestinal microflora, and alter the mucosal immune response (**Critchfield *et al.*, 2011**).

Mineral supplement

Disturbed cognitive function and concentration, mood changes, and slow growth may be associated with iron deficiency in autistic children. In addition, decreased serum iron levels can cause sleep and nervous system disorders which were significantly improved with oral iron supplements (**Dosman *et al.*, 2007**). Also, autistic patients appear to be at risk for zinc (Zn) deficiency (**Sweetman *et al.*, 2019**), Copper (Cu) toxicity and have low Zn/Cu ratio that predisposes the body to oxidative stress. Hence, zinc supplementation is required treatment for autism (**Isaacson *et al.*** 1996). Taking into consideration that it is important to estimate and follow the levels for both Cu and Zn together during Zn therapy because these two trace elements are antagonistic in function, and essential for living cells (**Bjørklund, 2013**). Moreover, selenium deficiency was documented in autistic children (**El- Ansary *et al.*, 2017**) therefore, it should be supplied.

Amino acid supplement

Amino acids play a crucial role in the brain as they are precursors to neurotransmitters or they behave as neurotransmitters themselves. Serotonin plays essential role in both brain and intestinal development. Since the brain-gut axis disturbances are a major complication in autistic children; serotonin may modulate these changes (**Margolis, 2017**). Tryptophan supplementation potentiates the production of serotonin. Taurine has antioxidant properties and is associated with improved visual symptoms. Also, L-carnosine has been shown to improve vocabulary, total score and behavior in autistic children (**Chez *et al.*, 2002**). It is obvious that the neurotransmitter imbalance in the central nervous system (CNS)could participate in autism pathophysiology. There was an increase in glutamate levels in autistic children as a result of the upregulation of glutaminergic gene expression. In this concern, the oral administration of N-acetylcysteine exerts an anti glutaminergic effect besides its antioxidant power through glutathione production (**Dean *et al.*, 2011**). Besides, the aforementioned potential role on mTOR that exerted by many amino acids.

Polyunsaturated fatty acids (PUFA) supplement

Phospholipids in the brain and retina are rich in PUFA, especially n-3 PUFA such as docosahexaenoic (DHA). DHA converted to oxylipins by 15-lipoxygenase. Oxylipins could regulate cell redox homeostasis and neurotransmitters signaling pathways. There is evidence that inadequate consumption of maternal DHA may be a risk of impaired CNS function. Also. DHA intake above the nutritional requirement may modify the risk of many CNS diseases (**Sun *et al*., 2017**). It was found that ASD populations have decreased DHA levels and, hence, n-3PUFA supplementation can noticeably improve ASD symptoms (**Mazahery *et al*., 2017**). In contrast, **Politi *et al.* (2008)** observed no significant improvement of disease severity and frequency, after omega-3supplementation in adult patients with ASD. On the contrary, **Cheng *et al.* (2017)** suggest that supplementation of omega 3 fatty acids may improve hyperactivity, lethargy, and stereotypy in ASD patients. Also, a comprehensive nutritional and dietary intervention with DHA and eicosapentaenoic acid (EPA), vitamin A, B complex, folic acid and coenzyme Q10 for autistic children, showed a significant improvement in autism symptoms and developmental age **(Adams *et al.*, 2018)**. Further trials are required to explore the potential advantages of omega 3 fatty acid supplementation in ASD patients.

Elimination diets

These diets were designed to reduce or even completely remove foods or food additives in ASD patients, they include:

Gluten-free diet and/or casein-free diet

Patients with ASD have gastrointestinal tract troubles that might be due to increased intestinal permeability. Digestion of casein and gluten generates peptides that can reach the bloodstream through the leaky gut and bind to the opioid receptors, causing deleterious CNS effects (**Reichelt *et al*., 1990). Hyman *et al*. (2016)** do not provide evidence to document the general use of the gluten-free /casein-free diet**.**

Ketogenic diet

It contains high fat, low carbohydrate, and low protein concentrations consequently provide about 90% of energy from fat. The ketogenic diet could reduce the symptoms in the autistic patients with a significant improvement in communication ability (**Evangeliou *et al*. 2003**). The ketogenic diets may improve social effect in children with ASD (**Lee *et al*., 2018**).

Specific carbohydrate diet

This diet mainly contains monosaccharides from fruits, honey or vegetables, while complex carbohydrates are limited. It is used to alleviate the malabsorption and growth of pathogenic intestinal microorganisms (**Gottschall, 2004). Barnhill *et al.* (2020)** declared at the 16-week intervention with the specific carbohydrate diet protocol was well tolerated in a 4-year-old child diagnosed with ASD and Fragile X syndrome, improving growth status, gastrointestinal symptoms, and behaviors.

Low oxalate diet

In autistic children, gastrointestinal dysfunction permits some substances like oxalate to cause abnormalities in their CNS. Autistic patients have high blood levels of oxalate about 3 fold the reference value with concomitant increased risk for ASD (**Konstantynowicz *et al.*, 2012**). Therefore, the autistic patients should restrict oxalate containing food like spinach, figs and green apples.

3. Conclusion

ASD accounts for substantial social and financial burden across the lifespan. The plausibility of the role mTOR signaling pathway of nutrition and environment as risk factors for ASD is growing. It is obvious that both factors appear to be causal and not co-founded. There is a link between autism and gastrointestinal disorders. Therefore, parents and their assistants should take into consideration the benefits of nutritional intervention for their autistic patients. Dietary supplements of the nutritional deficiencies of autistic patients are essential. Supplements of omega-3 fatty acid, amino acids, probiotics, minerals, and vitamins may be required in combination with medical and psychological treatments. Besides, a suitable elimination diet that were tailored to each patient according to one's symptoms may relief both autism symptoms and gastrointestinal disorders. It is desirable to continue future research into the relationship between ASD and maternal environmental pollutants' exposure and nutritional status. The reduction of environmental chemical exposures and considering nutrition as an important determinant in autism opens new avenues for lowering the risk of ASD. Large-scale epidemiological studies are needed to confirm the existing findings.

Declaration of interest

The authors have no conflicts of interest.

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Bisphenol A Analogues: A Brief Review of their Occurrence in Food, Biological Samples and Endocrine Effects

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Abstract

Bisphenol A (BPA) is the most well-known compound from the bisphenol family. There is increasing evidence that bisphenol BPA used in plastics, receipts, food packaging, and other products might be harmful to human health due to its actions as an endocrine-disrupting chemical, therefore BPA is being replaced by compounds very similar in structure, but data on the occurrence and effects of these BPA analogs are limited. Therefore, there is increasing concern regarding human exposure to bisphenol analogs (BPs) due to their widespread use and potential adverse effects. The main objective of this work was to investigate human exposure to BPs and the associated endocrine activities. We performed a literature review of the available research made in humans, in *in vivo* and in *vitro tests*. The findings support the idea that exposure to BPs may have an impact on human health, especially in terms of endocrine disruption.

1. Introduction

Endocrine disruptors are compounds that alter normal functioning of the endocrine system, and their bioaccumulation in humans may cause adverse health effects **(Andujar et al., 2019)**. Bisphenol A was first synthesized in 1905, with the condensation of phenol and acetone in the presence of acid as the catalyst **(Rykowska and Wasiak, 2006)**, afterward, its production levels increased, and nowadays BPA is one of the most extensively used bisphenols, mostly as a monomer in the production of polycarbonate plastics and epoxy resins **(Michałowicz, 2014)**. In recent years, there is increasing evidence of possible negative effects of bisphenol A (BPA) used in plastics, receipts, food packaging, and other products to human health due to its actions as an endocrine-disrupting chemical (EDC) **(Rochester, 2013; Rochester et. Bolden, 2015)**. Scientists, regulators, and the general public have raised concerns about the use of BPA, which has prompted the industry to seek alternative chemicals such as bisphenol AF (BPAF), bisphenol B (BPB), bisphenol F (BPF), and bisphenol S (BPS) (**Vandenberg et al. 2010; Rochester et. Bolden, 2015)**. Ideally, substitutes used to replace a chemical of concern should be inert, or at least far less toxic than the original chemical, but as seen in Figure 1, BPAF, BPB, BPF, and BPS are structural analogues to BPA, thus its effects in physiological systems may be similar (**Rochester et. Bolden, 2015)**. Although BPA alternatives have been largely used, so far, their toxicological information is limited **(Eladak et al., 2015)**. Given (anti-)estrogenic and (anti-)androgenic activities of BPA, several studies have demonstrated similar activities of BPA alternatives **(Rochester et Bolden, 2015)**.

2. Material and methods

A review of the available literature was conducted in October 2020. PubMed/Medline and Scopus databases were searched using the keywords “Bisphenol A analogues”, “Bisphenol A substitutes”, “hormone effect”, “endocrine disruption”, and “endocrine activity”. Data published between 2000 and 2020 were considered. We conducted a systematic review to identify the occurrence in food and biological samples, as well as adverse effects of bisphenols and their endocrine activities by focusing on animal models and *in vitro* mechanistic studies. After critical analysis of results, lines of evidence were built using a weight-of-evidence approach to establish a biologically plausible link.

3. Results and Discussion

Occurrence of Bisphenols

Bisphenol A (BPA) is one of the highest-volume chemicals used widely in the production of diverse consumer products, and its use has resulted in ubiquitous existence in the environment and organisms **(Zhang et al., 2018)**.

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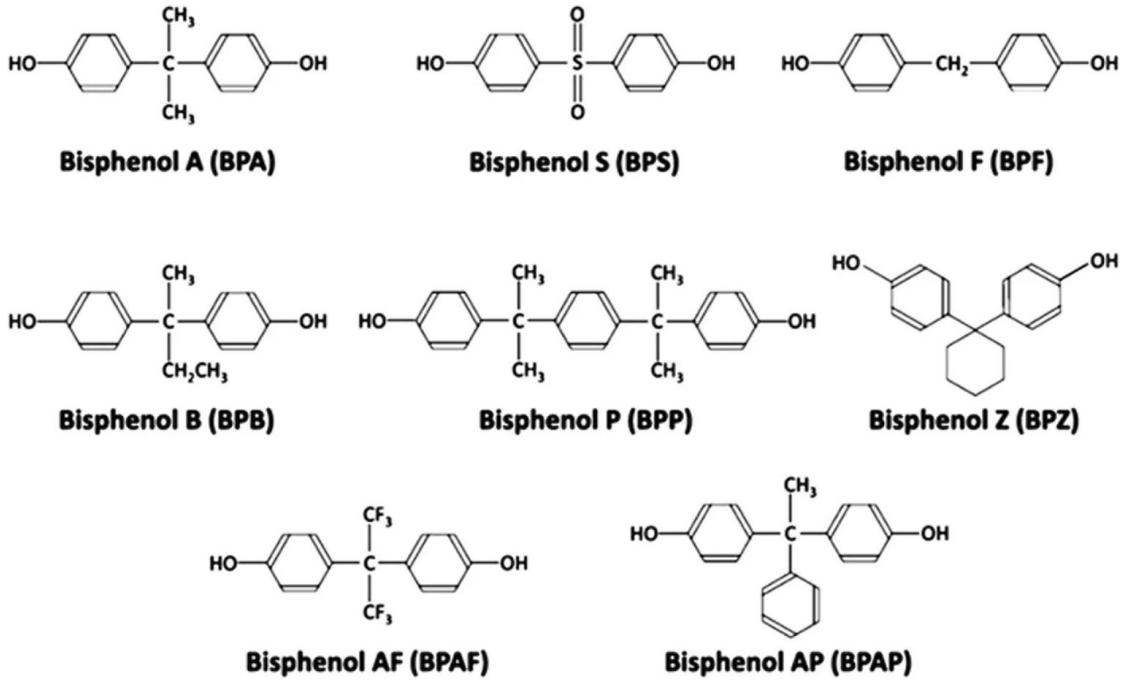

Figure 1. Chemical structure of bisphenol analogues. **(Yamazaki et al., 2015)**

The occurrence of BPA in more than 90% of 2,517 urine samples tested, with a geometric mean of 2.6 µg/L **(Calafat et al., 2008)**, in several studies of human biomonitoring, BPA has regularly been detected in urine, blood, milk, and other biological samples **(Azzouz et al., 2015; Covaci et al., 2015; Gramec-Skledar et Peterlin-Masic, 2016; Ye et al., 2015; Zimmers et al., 2014)**, which indicates the high human exposure. BPS and BPF have been detected in many everyday products, such as personal care products (e.g., body wash, hair care products, makeup, lotions, toothpaste) **(Liao and Kannan 2014)**, paper products (e.g., currency, flyers, tickets, mailing envelopes, airplane boarding passes) **(Liao et al. 2012c)**, and food (e.g., dairy products, meat and meat products, vegetables, canned foods, cereals) **(Liao and Kannan 2013)**. BPS, BPF, and BPA have been detected in indoor dust at the following concentrations: BPS, 0.34 µg/g; BPF, 0.054 µg/g; BPA, 1.33 µg/g **(Liao et al. 2012b)**. BPS and BPF have also been detected in surface water, sediment, and sewage effluent, generally at lower concentrations than BPA, but in the same order of magnitude **(Fromme et al. 2002; Song et al. 2014; Yang et al. 2014)**. In humans, BPS and BPF have been detected in urine at concentrations and frequencies comparable to BPA **(Liao et al. 2012a; Zhou et al. 2014)**. In urine samples from 100 American, nonoccupationally exposed adults, **Liao et al. (2012a)** found BPF in 55% of samples at concentrations up to 212 ng/mL, and BPS in 78% of samples at concentrations up to 12.3 ng/mL. BPA was found in 95% of the samples, with concentrations up to 37.7 ng/mL **(Rochester et Bolden, 2015)**. Little information is available on the occurrence of bisphenol analogues (BPs) in foods. In a study conducted in the United States, **Liao and Kannan (2013)** determined the prevalence of BPA, BPF, and BPS (N = 267) in nine food categories and found that 75% of the samples contained BPs, with total concentrations ranging from below the quantification limit to 1130 ng/g fresh weight (4.38 ng/g overall mean value). In preserved and ready-to-eat foods, the highest concentrations of BPF and bisphenol P (BPP) were 1130 ng/ g and 237 ng/g respectively. BPs in drinks and vegetables, by comparison, were detected at concentrations of 0.341 ng/g and 0.698 ng/g, respectively. In canned food, higher levels of individual and total BPs were found than in foods that came in containers of plastic, glass, or paper. In a study conducted in China, **Liao and Kannan (2014)** determined the presence of eight BPs (N = 289) in 13 food categories using high-performance liquid chromatography-tandem mass spectrometry. BPA and BPF were the most commonly observed BPs, detected at mean value concentrations of 4.94 ng/g and 2.50 ng/g fresh weight, respectively. In canned goods (27.0 ng/g), the highest overall concentration (sum of eight BPs) was observed, followed by fish and meat (16.5 ng/g), and drinks (15.6 ng/g). By comparison, milk and dairy products, cooking oils, and eggs (2-3 ng/g) had the lowest overall concentration. In canned foods (56.9 ng/g), higher total concentrations were found than in foods containing glass (0.43 ng/g), paper (11.9 ng/g), or plastic (6.40 ng/g). The presence of BPA analogs in canned vegetables, fruits, and soft drinks **(Gallart-Ayala et al., 2011a; Gallart-Ayala et al., 2011b)** and honey **(Cesen et al., 2016; Sadeghi et al., 2016)**, fish **(Sadeghi et al., 2016)** and mustard **(Zoller et al., 2016)** has been documented in many studies. Mustard is one of the most commonly used condiments in the world and is the main source of BPF in humans, in Europe and possibly worldwide, according to some authors **(Zoller et al., 2016)**. Although BPA is the most studied BP, BPs are widely used in epoxy coatings applied in drinking water delivery systems and have also been found in drinking water. BPs from coatings may be exposed to chemical oxidants (disinfectants), which, in comparison to the parent compounds, have the potential to form by-products with increased or decreased estrogenic activity **(Lane et al., 2015)**. In babies under six months of age, breast milk is the primary source of nutrition and can also be used as a surrogate for internal exposure levels in mothers and fetuses. In breast milk samples, BPA, BPF, BPS, and BPAF were found by **Niu et al. (2017)**, with BPA being the most abundant BP, followed by BPF. **Li et al., (2020)** detected BPs in 181 serum samples from pregnant Chinese women. Ten BPs, including bisphenol S (BPS), bisphenol F (BPF), bisphenol AF (BPAF), bisphenol B (BPB), bisphenol P (BPP), bisphenol Z (BPZ), bisphenol AP (BPAP), tetrabromobisphenol A (TBBPA), tetrabromobisphenol S (TBBPS), and tetrachlorobisphenol A (TCBPA), were positively identified and quantified in serum samples with total BP concentrations (sum of bisphenols: ∑BPs) of 0-144 ng/mL.

Adverse Effects of Bisphenols

The presence of BPA analogues in food, environmental, and human biological samples indicates that it could affect the body. The most widely studied effect of BPA is estrogen activity, and its effects on other hormonal receptors have also been reported **(Gramec-Skledar et Peterlin-Masic, 2016)**. However, limited studies have confirmed that BPs have related BPA-like endocrine-disrupting activities **(Liao et al., 2012)**. The estrogenicity of BPs was first reported in 1998 in cultures of the human breast cancer cell line MCF7 using an E-SCREEN assay **(Perez et al., 1998)**. Later, in 2002, by evaluating the induction of pS2 (mRNA and protein) and progesterone receptors as well as the expression of the luciferase reporter gene transfected into MVLN cells, the effects of these chemicals on the expression of estrogen-controlled genes were demonstrated **(Rivas et al., 2002).** The estrogenic effect of certain BPs has also been reported to be higher than that of BPA **(Cao et al., 2017)**. BPS, for example, has a higher hormonal activity, which can probably be due to its heavy polarity and the presence of the sulfonyl group **(Caballero-Casero et al., 2016; Gallart-Ayala et al., 2011b)**, as well as to its thermal stability and light resistance **(Deceuninck et al., 2015; García-Córcoles et al., 2018)**. In addition, by inducing the proliferation and migration of MCF-7 clonal cells, BPS and BPF have been shown to be involved in the development of breast cancer **(Kim et al., 2017)**. **Van Leeuwen et al. (2019)** recently stated that most in vitro studies of BPA analogs have comparable or higher estrogenic activity than BPA, as well as greater antiandrogenic properties. Other BPA analogues have shown both antiestrogenic and antiandrogenic activity. The most studied bisphenol analogues are BPS and BPF. In a systematic review of 32 studies (25 *in vitro* and 7 *in vivo*), the potency of BPF and BPS was found to be in the same order of magnitude as that of BPA and to have comparable hormonal effects **(Rochester et Bolden 2015)**. In addition, the study found that BPS and BPF had hormonal effects, such as changes in organ weights and levels of enzyme expression, beyond those of BPA. The authors concluded that BPS and BPF tended to have similar potency and mechanisms of action to BPA, which had similar effects on health. In terms of their toxicological profiles, including metabolic, carcinogenic, and reproductive effects, as well as oxidative stress and DNA damage, other authors have also reported on the similarity between BPS and BPF and BPA **(Rosenmai et al., 2014; Rochester et Bolden 2015; Roelofs et al., 2015; Gallo et al., 2017)**. Adverse reproductive consequences secondary to exposure to BPA analogs, such as decreased sperm and oocyte production and steroidogenesis, have also been indicated in some studies in animal models **(Siracusa et al., 2018)**. BPS was shown to reduce the weight of gonads and alter plasma estrogen and testosterone in zebrafish, as well as to decrease egg development and hatchability, with longer hatching times, and to increase embryo malformations **(Qui et al., 2019)**. **Shi et al. (2018)** have shown that prenatal exposure to physiologically relevant doses of BPA analogues is likely to affect male reproductive functions due to a spermatogenic defect in the developing testis. The effect of low dose chronic exposure to BPB, BPF, and BPS on hypothalamo-pituitary-testicular behaviors in adult rats was shown by **Ullah et al. (2019)**. **Shi et al. (2019)** concluded that the initiation of puberty was accelerated by prenatal exposure to bisphenols, and the female mice had fertility problems, irregular estrous cyclicity, and dysregulated expression of steroidogenic enzymes, especially at lower doses. **Kolla et al., (2018)** compared the exposure effect of BPA and BPS on female mouse mammary gland development during the perinatal phase. Age-specific and dose-specific effects of BPS that were different from the effects of BPA were observed in the study. Furthermore, using L1 larvae of the *Caenorhabditis elegans* model animal, **Zhou (2018)** measured low-concentration BPS toxicity. Multiple indicators have been examined at physiological, biochemical, and molecular levels, the overall results showed that BPS was less noxious compared with the effects of BPA, indicating that individual bisphenols could have specific effects. **Eladak et al. (2015)** conducted research, which showed that 10 nmol/L BPS and BPF can minimize the secretion of basal testosterone by fetal human and mouse testicles. **Desdoits-Lethimonier et al., (2017)** showed that BPE, BPF, BPB, and BADGE exhibited antiandrogenic properties in adult human testes using an *ex vivo* culture system. In addition, BPA, BPAF, BPB, BPF, BPS, and bisphenol Z (BPZ) have been found to alter thyroid endocrine system function in a study conducted on the GH3 rat cell line, which appears to be increased by 17β-estradiol **(Lee et al., 2018)**. **Serra et al., (2019)** reported that existing information on BPB's estrogenic activity and inhibition of testosterone production is similar to BPA's endocrine activity.

3. Conclusion

There is increasing concern regarding human exposure to bisphenol analogues (BPs) due to their widespread use and potential adverse effects. It is not surprising that these replacements also pose a danger to wildlife and human health, considering the similarities between BPA and BPA analogues in terms of their metabolism and behavior, including hormonal effects beyond those of BPA. Regulations for the safety evaluation of consumer goods should be expanded to include all substances in the same category of chemicals. Futhermore, more work is required to find chemical alternatives without harmful health effects, as recommended by various researchers. The trend towards replacement of BPA analogs in consumer goods, especially food contact materials, should be exercised with caution and should include effective and frequent monitoring to assess their effects on human health.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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